Direct Visualization of Horizontal Gene Transfer in Cotton Plants

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Plant mitochondrial and chloroplast genes that underwent horizontal transfer have been identified by parasite and grafting systems, respectively. Here, we directly observed 3 horizontal gene transfer (HGT) events in the 45-second auxillary shoots of grafted cotton plants (Gossypium barbadense and Gossypium hirsutum) after extirpating the first axillary bud. The second auxillary shoots showed phenotypic variations in cotton flowers and seeds that were evidence of spontaneous development from cells in the grafting site. As the progeny segregated and did not show stable inheritance across 3 generations, inheritance of traits in our study differed from the stable heredity of HGT plants in previous studies. In those studies, plants were artificially regenerated from the graft junctions, and inheritance involved only the movement of chloroplast DNA or genomic material between cells. Our findings may provide a feasible method to enhance plant breeding and the study of HGT.

**Subject areas:** Gene action; Regulation and transmission

**Key words:** horizontal gene transfer, grafting cotton, axillary bud

Horizontal gene transfer (HGT) is the process of genetic movement between species and plays a role in their evolution. In prokaryotes and eukaryotes, HGT has been well documented and is still being extensively studied. Most HGT events were detected by phylogenetic analysis (Davis and Wurdack 2004; Mower et al. 2004; Yoshida et al. 2010). Plants as both donors and recipients can participate in the process of HGT, as can prokaryotes. Several recent studies suggested that HGT events between plants could occur via a plant host–parasite or grafting system (Davis and Wurdack 2004; Mower et al. 2004; Stegemann and Bock 2009; Yoshida et al. 2010; Stegemann et al. 2012; Thyssen et al. 2012), indicating cell–cell contact was a requirement for HGT. The plant host–parasite system indicated that the mitochondrial genome was unusually active in HGT compared with that of other organelles (Davis and Wurdack 2004; Mower et al. 2004). However, HGT in nuclear genes has rarely been reported; only a few studies on nuclear HGT were conducted using parasitic plants to examine gene phylogenies (Yoshida et al. 2010). Furthermore, the plant grafting systems presented clear evidence of HGT but only at the immediate graft site, and chloroplast DNA or genomes of HGT were merely identified (Stegemann and Bock 2009; Stegemann et al. 2012; Thyssen et al. 2012).

Plant parasite and grafting systems offer 2 avenues to further investigate HGT (Davis and Wurdack 2004; Mower et al. 2004; Stegemann and Bock 2009; Yoshida et al. 2010; Stegemann et al. 2012; Thyssen et al. 2012). The plant cell has 3 DNA-containing cellular compartments: the nucleus, plastids, and mitochondria. Grafting was successfully used as a potent method to detect the occurrence of HGT, but only focusing on chloroplast DNA (Stegemann and Bock 2009; Stegemann et al. 2012; Thyssen et al. 2012). To date, nuclear and mitochondrial gene transfers in grafting sites have not been reported, but they may occur.

To find new evidence regarding plant–plant HGT and to better understand the underlying mechanism, it is important to develop a new genetic experimental system to facilitate the study of HGT. In this study, we obtained visual evidence of HGT in grafted cotton, which will help us to study the underlying genetic and molecular mechanisms of plant–plant HGT and promote new approaches for plant breeding.

**Materials and Methods**

We selected Sea Island cotton (Gossypium barbadense L., 2N = 52, AADD) (line Hai7124, characterized by naked seeds, yellow pollen, bright yellow petals, red spots on petals, and non-Bt) and upland cotton (Gossypium hirsutum, 2N = 52, AADD) (cultivar Lu21, characterized by fuzzy seeds, pale
cream petals, absence of petal spots, and Bt). These 2 lines of *Gossypium* had several differences in flower traits and plant architecture (Figure 1). The heterograft plants were established by transplanting upland cotton scions onto Sea Island cotton rootstocks under aseptic conditions before transplanting into the field. In addition, a control group of cotton homografts was generated using the same species. Comparisons were performed between the plants of the same grafted combination, between the axillary shoot of first emergence and the second axillary shoot after extirpating the first axillary shoot. The grafts were transplanted into the field 15 days after grafting.

After the grafted cotton plants were grown for several days, the axillary bud was located between a leaf and the shoot axis, which developed from the rootstock at the grafting junction. Because axillary shoot meristems can develop from cells at the base of the subtending leaf and may initiate at the same time as the subtending leaf, we hypothesized that no reciprocal genetic transfers occurred between the scion and rootstock in the first axillary shoots from the grafting site.

To test our hypothesis, we performed contrast experiments. The axillary shoots were reserved at first emergence as the control, and after the axillary shoot of first emergence had been extirpated, the second axillary shoot spontaneously initiated in the same growing point in many cases. Once initiated, the axillary shoot meristems may develop into branches, which could flower and fruit in the axillary branching.

### Results and Discussion

Of the 45 second axillary shoots that we examined during the flowering time, there appeared to be 3 HGT events that showed difference in petal color and petal spot from those of the rootstock and scion (Table 1, Figure 1). However, no phenotypic changes were found in the first axillary shoots and homografts (Table 1).

On HGT branches, the flowers had light yellow petals with reddish spots on the basal part (Figure 1). Moreover, the seeds were not fuzzy but had numerous short fibers and gray testa, a color midway between those of Sea Island cotton and upland cotton (Figure 2). After the seeds were sowed, the progeny plants presented a segregating population, and the flowers exhibited diverse combinations of petal color (yellow, light yellow, and pale cream), petal spot (red, reddish, and no spot), and anther color (yellow and yellow cream). Further, in the next generation, partial HGT plants showed malformed or male sterile flowers, whereas other plants showed multiple flower traits.

The phenotypic variations were due to endogenous DNA transmission from scion to rootstock, according to the segregating progeny. Moreover, these genetic variations from the second natural axillary shoots were not stably inherited across 3 generations but were inherited differently from the plants that were artificially regenerated from the graft junctions. Inheritance involved the movement of chloroplast DNA or genome between cells (Stegemann and Bock 2009; Stegemann et al. 2012; Thyssen et al. 2012). It is not clear whether genetic materials are not fully transmitted before the graft junctions are used in tissue cultures as a result of shorter several days after grafting (Stegemann and Bock 2009; Stegemann et al. 2012; Thyssen et al. 2012). It is also possible that the regeneration system changes the existing epigenetic mode, perhaps through chromatin remodeling (Miguel and Marum 2011); in addition, some DNA or organelles plants that underwent HGT may be lost during the tissue culture processes. Another possible cause for the genetic variations is the interaction between the cell organelles of plants that underwent HGT and the nucleus, although the HGT axillary branches were indeed heterozygous and segregated in the progeny.

### Table 1  Phenotypic changes in the axillary shoots in the grafting junction of heterografts and homografts

<table>
<thead>
<tr>
<th>Grafts (scion/stock)</th>
<th>Grafted combinations with the axillary shoot of first emergence</th>
<th>Grafted combinations with the second axillary shoot after extirpating the first axillary bud</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total no. of grafts</td>
<td>No. of axillary shoots with altered phenotype after grafting</td>
</tr>
<tr>
<td>Lu21/Hai?124</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>Lu21/Lu21</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>Hai?124/Hai?124</td>
<td>37</td>
<td>0</td>
</tr>
</tbody>
</table>
The horizontal transfer of nuclear Bt genes, which accounted for a very small ratio of the nuclear genome, was not observed in the second axillary branches. It is likely that transfer of nuclear DNA was random or nonfunctional. Although we could not identify the source (nucleus, chloroplast, or mitochondria) for the genetic material involved in HGT, this observation provides a possible path for creating genetic variations in higher plants characterized by axillary branching, such as tomato. This path may be especially valuable in sexually incompatible species and may be of use in plant breeding and the study of HGT as a new experimental system.

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**References**


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