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Arabidopsis thaliana as a Model System for Graft Union Development in Homografts and Heterografts

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Abstract Homografting of *Arabidopsis thaliana* scions on stocks of *A. thaliana* and heterografting on other species were used to study the compatibility and the ontogeny of graft union formation. Highly compatible homografting with scions of young leafy inflorescence stems was obtained on stocks of inflorescence stems growing from large 3-month-old *A. thaliana* plants. Histologic analysis revealed four developmental stages of graft union formation in *Arabidopsis* homografting: (1) development of a necrotic layer, (2) callus proliferation in the grafted scion, (3) differentiation of new vascular tissues within the scion, and (4) a full vascular graft union formation between the scion and the stock. Vascular connections were formed within the callus bridge between rootstocks and scions 15 days after grafting. Heterografts of *Arabidopsis* on two members of Brassicaceae, cabbage (*Brassica*) and radish (*Raphanus*), showed partial incompatible interaction with a lower level of vascular differentiation. *Arabidopsis* grafting on tomato (Solanaceae) rootstock showed complete incompatibility and limited noncontinuous differentiation of new vascular tissues that did not cross the scion/stock boundary. Although lacking scion/stock vascular connections, *Arabidopsis* scions grafted onto tomato rootstock flowered and produced seeds. This may indicate some nonvascular functional connections between the two plants, probably of parenchyma cells, further emphasizing the usefulness of *Arabidopsis* as a model plant for studying various levels of the complicated scion/stock relationships expressed in grafting biology. Experiments with dye transport in the xylem showed that although in general there was an agreement between the histologic study and dye transport, in *Arabidopsis* homografts water transport frequency was lower than functional and histologic compatability. We conclude that homografting and heterografting of *Arabidopsis* inflorescence stems is a convenient and reproducible method for studying the fundamental cellular genetic and molecular aspects of grafting biology.

Keywords *Arabidopsis thaliana* · Inflorescence stems · Grafting · Incompatibility · Vascular differentiation

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Introduction

Grafting is an ancient cloning method through which desirable genotypes of fruit trees that do not have natural adventitious rooting capacity, or of those that may benefit from a specific stock-scion combination, are propagated. For over two millennia, grafted varieties of fruit trees have been an important source for human nutrition (Zohary and Hopf 2000). Many ornamental varieties and forest trees are also propagated by grafting (Hartmann and Kester 1975). Recently, grafting has also become common in annual dicotyledonous field crops, leading to superior resistance of grafted combinations to pathogens and abiotic factors. For example, in Japan, where agricultural land use is intensive, almost 95% of watermelons (*Citrullus lamanis*), oriental melons (*Cucumis melo* var. *makuwa*), greenhouse cucumbers (*Cucumis sativus*), and various Solanaceous crops are grafted before being transplanted to the field or greenhouse

(Lee 1994; Edelstein and others 1999; Lee and Oda 2003). There are many methods of grafting plants, but all involve the joining of a scion of one plant with the rootstock of another. Grafting methods include apical-wedge grafting, whip-and-tongue grafting, splice grafting, flat grafting, saddle grafting, bud grafting, hole insertion grafting, tongue approach grafting, and cleft grafting (Toogood and Anderson 1999).

The agricultural use of grafting is considerably restricted to closely related taxa because of problems of incompatibility. The biological nature of this grafting incompatibility is actually not known. The ability to overcome grafting incompatibility would allow us to exploit grafting combinations that are currently impossible and would therefore be of great benefit to agriculture. *Arabidopsis thaliana* seems to be the best vehicle for rapid progress in this specific area.

Grafting has been used to study different aspects of plant biology including plant pathology (Bertaccini and Bellardi 1992; Cohen and others 2002), mineral nutrition (Jayawickrama and others 1992), apical dominance (Mapelli and Kinet 1992), nodulation (Pedalino and others 1992), flowering (Zeevaart 1958; Lifschitz and others 2006; Corbesier and others 2007), dwarfing (White and others 1992), characterization of mutants (Tsukaya and others 1993), and hormone action (Proebsting and others 1992). Grafting also provides an experimental means to juxtapose diverse genotypes and test for transport of signals or metabolites (Turnbull and others 2002).

Grafting experiments in combination with external hormone application have been used to develop fundamental knowledge related to the developmental-physiologic aspects of grafting. A major finding of these studies was that both stages of graft union formation, namely, callus proliferation and vascular differentiation, could be modulated by auxins (Sachs 1981). If auxin sources such as shoot tips and leaves were removed, then the formation of callus and vascular strands were reduced at the base of the scion. This suggests that in intact grafts the formation of callus and vascular connections is highly influenced by the flow or accumulation of auxins at the base of the scion, which is consistent with the theory of the basipetal polar flow of auxin (Sachs 1981). The presence and indispensability of auxin at the base of the scion for graft union formation are supported by the general observations that scions form more callus than stocks and that the application of exogenous auxin to scions before grafting improves grafting success in several scion/stock combinations (Wetmore and Rier 1963; Sachs 1993).

Grafting technique has already been demonstrated in *A. thaliana* (Tsukaya and others 1993; Rhee and Somerville 1995; Turnbull and others 2002). Grafting of *Arabidopsis* has been used to examine a wide range of developmental and physiologic processes such as long-distance signaling (Turnbull and others 2002; Booker and others 2003, 2005; Sorefan and others 2003; Nelson 2004; Lifschitz and others 2006; Corbesier and others 2007), the role of plant hormonal signaling in the regulation of shoot branching (Leyser 2003), floral development (An and others 2004), understanding the mechanism of systemic acquired resistance (Dong 2001, 2004), and post-transcriptional gene-silencing pathways (Rovere and others 2002). The present study focuses on the biology of graft union formation using *Arabidopsis thaliana* as a model plant. Establishing a system that expresses various levels of graft compatibility and incompatibility with *A. thaliana* will facilitate the study of cellular, molecular, and genetic processes and factors involved in this important agricultural technique, to a greater extent than any other model system.

The specific objectives of this study were (1) to characterize the developmental stages of graft union formation of inflorescence stems of *A. thaliana* and (2) to establish plant models for various levels of graft incompatibility with *A. thaliana*, to be used later in the identification and study of genes involved in graft formation and in incompatibility.

Materials and Methods

Plant Materials and Growth Conditions

Seeds of *Arabidopsis thaliana* var. Columbia were germinated in a growth chamber under short-day conditions (9 h light/15 h dark, 22 ± 1°C, 45 μIS^{-1} m^{-2}). Several dozen single, 7-week-old rosettes were transferred to 1.7-L pots, 30–40 mm in diameter, filled with a mixture of peat/tuff/perlite (40%/40%/20% v/v/v), fertilized once a week with Osmocot (NPK), and irrigated twice a week. Short-day regime (9 h light/15 h dark, 22 ± 1°C) was maintained for 2 months until the rosette leaves filled the pot area, after which the plants were transferred to long-day conditions (12 h light/12 h dark) to stimulate flowering. Large rosettes of *A. thaliana* were induced by repeated excisions of inflorescences (Lev-Yadun 1994). Inflorescence stems that developed from these large rosettes were used for further experiments.

Seedlings of *Brassica oleracea* cv. capitata cultivar OS (cabbage), *Raphanus sativus* cultivar Gloriette (radish), and *Solanum lycopersicum* cultivar Camelia 819 (tomato) were germinated in a controlled environment chamber at a day/night temperature of 25/22°C, under 12 h light/12 h dark conditions. The seedlings developed in 1.7-L pots filled with a mixture of peat/tuff/perlite (40%/40%/20% v/v/v), fertilized once a week with Osmocot (NPK) and irrigated twice a week.

Grafting Experiments

Several preliminary sets of grafting experiments were carried out using *A. thaliana* var. Columbia. Grafting was done using young inflorescence stems developing from large 3-month-old *A. thaliana* plants. Young inflorescence stems of uniform age, length, and diameter (as described below) were selected and used as rootstocks and scions in the *Arabidopsis* homografting. During these preliminary experiments, we defined the optimal grafting method, grafting location within the inflorescence stems, and timing for grafting.

In our previous related study on wound healing in *A. thaliana* inflorescence stems (Flaishman and others 2003), we found that regular regenerative xylem formation following wounding occurred only in young inflorescence stems, especially in their lower part. Therefore, we used only the lower part of 1- to 2-day-old inflorescence stems as scions in the grafting experiments. Inflorescence stems emerge as small buds, hidden among the large and dense rosette leaves. They are too fragile to handle until they reach a size of about 2 cm, which is attained 1–2 days after they emerge under the growth conditions used in these experiments. Both wedge and horizontal grafts were tested in preliminary experiments to determine the optimal method of grafting. Because inflorescence stems that were too thin often broke off when cut with a razor blade, only the more robust, 2–3-mm-thick inflorescence stems were used for grafting experiments. In addition, several devices were tested to hold both partners during union formation. The use of thin glass sticks (Figure 1C) was selected as the best method for holding the stems.

Twenty-five to 35 days after germination, cabbage, radish, and tomato scions were homografted on their own rootstock, and *Arabidopsis* scions from the large rosettes were heretografted on top of cabbage, radish, and tomato using the wedge grafting procedure. Grafted plants were covered with a transparent plastic bag for 5 days to increase relative humidity and minimize leaf dehydration. Ten to 20 plants were used for each set of grafting treatment and the grafting experiments were repeated three times. The percentages of successful and unsuccessful grafts were determined 25–30 days after grafting. Grafting was considered successful when scions remained alive on top of the rootstock. Additional grafting experiments were conducted to study water flow in the grafted plants (see below).

Histology of Graft Development

The sequence of anatomical changes that take place during graft union formation was examined by harvesting 12 *Arabidopsis* homografts on days 3, 6, 9, 12, 15, 18, 21, and 25 after grafting. In addition, six cabbage, radish, and tomato homografts were harvested on days 3, 9, 15, 21, and 27 after grafting. Furthermore, the histologic aspects of heterografts of *Arabidopsis* on cabbage, radish, and tomato were studied by harvesting six heterografts on days 15, 21, 24, and 30 after grafting. Specimens were trimmed to 5 mm above and 5 mm below the graft union, fixed for 7 days in FAA, and dehydrated through a series of ethyl alcohol dilutions (30, 50, 70, 90, and 96%) and three times in absolute ethanol for 6 h per stage. Tissue samples were then embedded in paraffin (TissuePrep, Fisher Scientific, Fairlawn, NJ) in an oven at 55 ± 2°C. Longitudinal serial sections, 10 μm thick, were prepared using a rotary microtome (American Optical model 820). Tissue specimens were spread on glass slides with a drop of Haupt adhesive (Carolina Biological Supply, Burlington, NC), which was applied on a slide and spread evenly across it. Two drops of floating solution (3%, v/v, formaldehyde) were added before the slides were placed on a hot plate and maintained at 39°C. Plant samples were stained with aqueous Safranin-O and fast-green (Sigma Chemical, St. Louis, MO) and mounted with Permount (Fisher Scientific). Finally, the slides were examined under a Leica

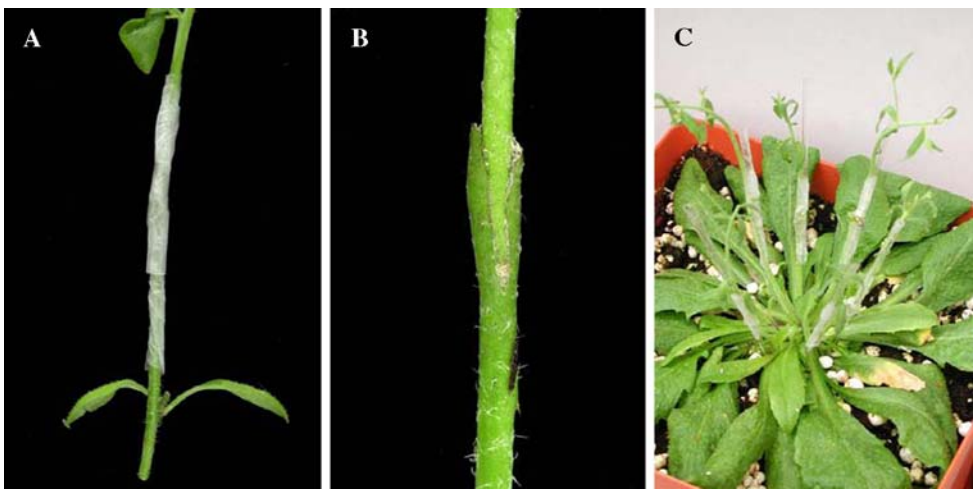

Fig. 1 Homologous wedge grafting in *Arabidopsis*. (**A**) A grafted plant immediately after grafting (the grafted area is covered with parafilm). (**B**) A grafted plant 3 days after grafting (parafilm removed for photography). (**C**) Several grafted inflorescences in a single potted plant 6 days after grafting

DMLB light microscope and representative sections were photographed. Using the serial sections, we examined the whole width of stock and scion and determined whether graft union occurred, where it occurred within the tissues, and the proportion of successful grafting.

Visualizing Water-Conduction Pathways in Grafted Plants

To visualize water-conduction pathways in the different grafts, we introduced aqueous solutions of acid fuchsin into the vascular system of the plants by submerging the roots into the aqueous solution of 0.5% (w/v) acid fuchsin (Sigma) dye at room temperature. The solution was allowed to flow into the vascular system for 50 min. The transportation of the dye above the grafted part was inspected by cutting two discs (2 mm thick) in the scion and microscopically examining the staining by dye on the surface of each disc.

Results

Grafting and Growth

Successful grafting of *A. thaliana* inflorescence stems varied according to the type of graft tested. In general, the wedge grafting method was significantly more successful (91% viability) than the horizontal grafting method (30% viability). In the wedge grafts, no problems were encountered during the union formation provided the contact surfaces were tight. Less than 10% of graft failure occurred in the wedge grafts because of mismatched tissues between the scion and the rootstock. Horizontal grafting, a method used previously by Rhee and Somerville (1995) for *Arabidopsis*, was more difficult to perform and had a much lower rate of success because of mismatched tissue contact between the scion and the rootstock. In successful grafts, the re-establishment of growth by scions became evident 5–10 days after grafting (Figure 1). Given its clear superiority to all other methods, the wedge method was used in all subsequent experiments.

Using the wedge grafting method, homografts of *A. thaliana*, cabbage, radish, and tomato as well as herografts of *A. thaliana* scions on rootstocks of cabbage, radish, and tomato were performed. Table 1 summarizes the successful homografting and heterografting between *A. thaliana* and members of Brassicaceae (cabbage and radish) or tomato, 25–30 days after grafting in the greenhouse. The fraction of successful homografting varied between the different species. Although *Arabidopsis* and tomato homografting showed very high success rates, homografts of cabbage and radish had lower ratios of successful grafting (Table 1). Heterografting with *Arabidopsis* used as a scion on cabbage, radish, and tomato rootstocks showed lower percentages of successful grafting relative to *Arabidopsis* homografts (Table 1). Successful grafting of *Arabidopsis* scions on other species resulted in physiologically functional combinations. This was evident from the development and flowering of the scions, which are processes that require the transport of water, minerals, hormones, and probably other signals.

Table 1 Ratio of Successful Homografting and Heterografting Between *Arabidopsis*, Members of Brassicaceae, and Tomato

Scions/Rootstock	Number of grafts	Successful grafting (%)
Arabidopsis/Arabidopsis	140	80-95
Brassica/Brassica	53	68
Raphanus/Raphanus	53	23
Tomato/tomato	51	100
Arabidopsis/Brassica	56	67
Arabidopsis/Raphanus	59	22
Arabidopsis/tomato	53	49

Grafting was considered successful when scions remained alive 25–30 days after grafting

Anatomy of Graft Union Formation in *Arabidopsis thaliana* Homografting

The histology of graft union formation in *A. thaliana* homografting showed four developmental stages of graft union formation: (1) development of a necrotic layer, (2) callus proliferation in the grafted scion, (3) differentiation of new vascular tissues within the scion, and (4) a full vascular graft union formation between the scion and the rootstock. During the first three days after lining-up the rootstock and scion, a distinct shrunken, darkly stained necrotic cell layer became visible in some sectors of the contact zone between the scion and the rootstock, although most of the contact zone remained parenchymatic (Figure 2A).

The first evidence of cell division became apparent between days 3 and 6 after grafting as callus developed within the scion at the interface between the scion and the rootstock. Areas of parenchyma cells adjacent to the necrotic layer started to divide at different points along the inner surface of the wounded tissues, exclusively on the scion part, forming a callus (Figure 2B). During the early stages of callus formation, the dividing cells were axially elongated but remained relatively small compared to other typical parenchyma cells belonging to the scion and rootstock tissues. By day 9, intense cell division activity occurred on both the rootstock and the scion at the graft interface as indicated not only by callus proliferation across the graft contact zone but also by the disappearance of the necrotic zone. Differentiation of vascular tissues (stage 3)

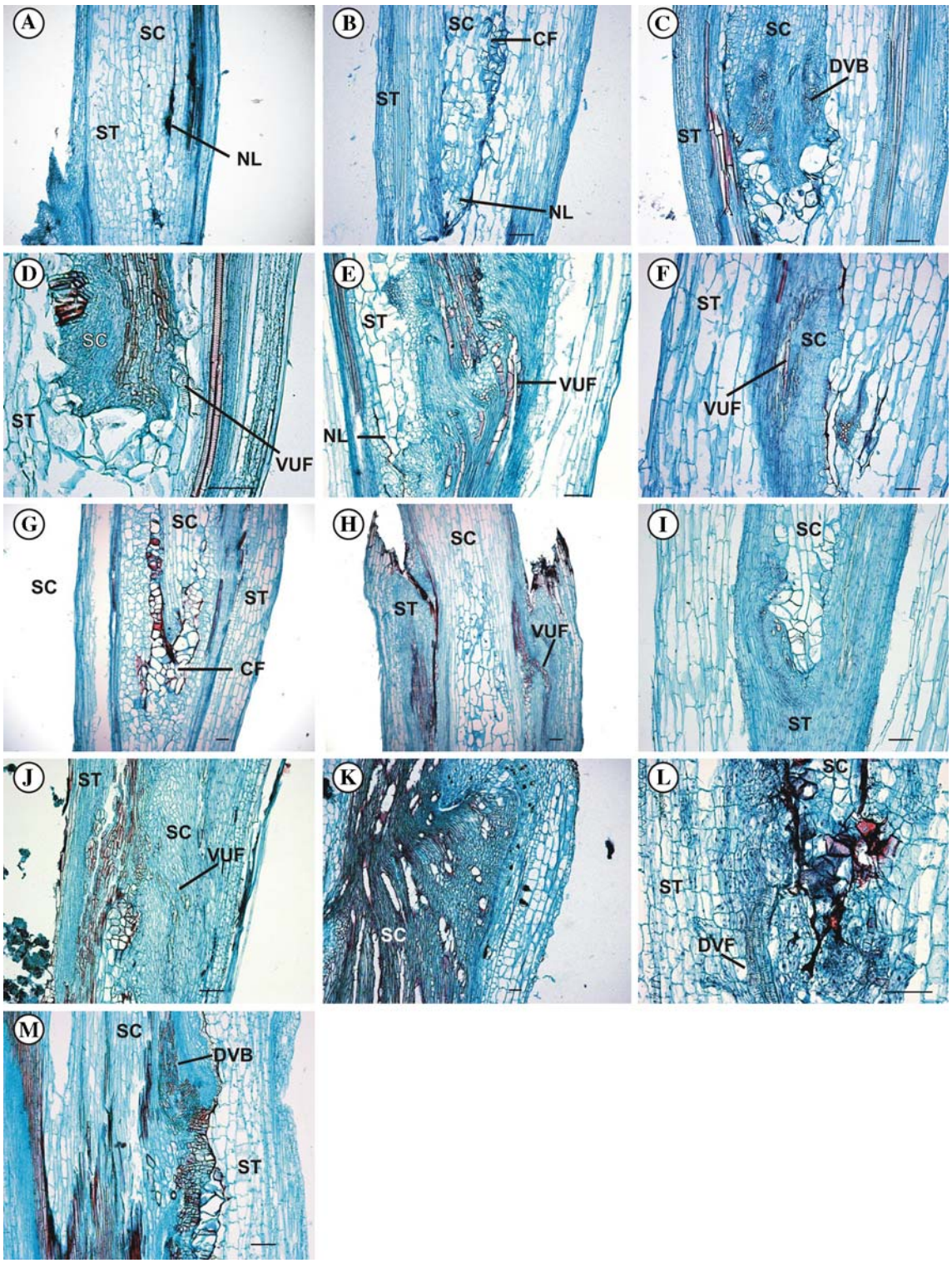

Fig. 2 Compatible and incompatible grafting interactions. Homografts in *Arabidopsis* (**A**, after 3 days; **B**, after 6 days; **C**, after 9 days; **D**, after 15 days). Homografts in cabbage (**E**, after 25 days) and radish (**F**, after 25 days). Homografts in tomato (**K**, after 30 days). Heterografts of *Arabidopsis* on cabbage (**G**, **H**, after 30 days), radish (**I**, **J**, after 30 days), and tomato (**L**, **M**, after 25 and 30 days, respectively). Scale bar = 100 μm. ST, rootstock; SC, scion; NL, necrotic layer; CF, callus formation; DVB, developing vascular bundles; VUF, vascular union formation

was obvious after day 9 (Figure 2C). In the active meristematic zone, advanced stages of vascular differentiation were visible within the scion but a full graft union with the rootstock was not yet formed. A well-developed vascular graft union was observed only 15 days after grafting (Figure 2D), when new vessel members were seen to unite across the interface zone between the scion and the rootstock. At this stage, the necrotic layer was only slightly visible along the wounded zone. The epidermis layers of the rootstock and the scion became continuous at the upper union zone 21 days after grafting.

Heterografts Between Members of Brassicaceae

To examine graft compatibility between members of different genera of Brassicaceae, we grafted *Arabidopsis thaliana* var. Colombia scions on cabbage and radish rootstocks, with the controls being homografts of *Arabidopsis* on *Arabidopsis*, cabbage on cabbage and radish on radish. The rates of successful grafting are presented in Table 1. Generally, homografts were more successful than heterografts. Interestingly the percentage of success on *Brassica* rootstock and *Raphanus* rootstock were identical regardless of whether they were in homografts or heterografts. Although not proven, a rootstock effect cannot be ignored.

The rate of success was not the only distinguishing parameter between the grafted groups. Based on our preliminary experiments on the kinetics of graft development using *Arabidopsis* homografts, we determined the end of the experiment and the dates of sampling (Figure 2A–D). Accordingly, we sampled the heterografts 15, 21, 24 and 30 days after the date of grafting. Homograft controls of cabbage on cabbage and radish on radish formed well-developed graft unions (Figure 2E, F). In successful cases of grafting *Arabidopsis* scions on either the cabbage or the radish rootstocks, the histologic outcome was similar: in all samples the lower part of the scion showed callus formation (Figure 2G, H) and, in some cases, vessel members were formed only at the lower part of the scion/stock contact zone, although neither vascular bridge nor full graft union was visible. However, in 83% of both the cabbage and the radish heterografts, graft union formation commonly occurred in the upper parts of the heterografts, spanning most of the contact zone between the *Arabidopsis* scion and the rootstock (Figure 2I, J).

Heterografts Between Tomato Rootstock and *Arabidopsis* Scion

In addition to grafting experiments within Brassicaceae, we conducted experiments with grafting tomato, a more distant taxon belonging to Solanaceae. Tomato homografts produced broad and strong graft unions, characterized by secondary wood (Figure 2K). In contrast, in heterografts involving the two families, the tomato rootstocks produced considerable amounts of regenerative xylem that approached the contact zone with the *Arabidopsis* scion (Figure 2L). The *Arabidopsis* scion produced new vascular elements as well, but these did not cross the scion/rootstock border and therefore no graft union was formed (Figure 2M). In response to grafting, one or two cell layers in the tomato stock, located close to the scion/stock contact region, became enriched with material that stained dark red, similar to polyphenoles. It appears that this material may have restricted or at least been involved in preventing vascular bridge formation between the two species—a typical incompatible response (Figure 2L, M). Despite the lack of vascular connections, *Arabidopsis* scions grafted onto tomato rootstock were able to flower and develop seeds. This may indicate some level of nonvascular functional connections between the two plants, probably composed of parenchyma cells.

Visualizing Water-Conduction Pathways in Grafted Plants

Table 2 summarizes the successful water-conduction pathways in homografting and heterografting of *A. thaliana* and members of Brassicaceae (cabbage and radish) or tomato, as evident by stain transport 17–25 days after grafting in the greenhouse. The fraction of successful water conduction varied between the different species. Surprisingly, homografts in *Arabidopsis* transported dye in a much lower proportion than that indicated by histologic examination and by the functionality of the grafts that grew well, flowered and set seeds. Water conduction in the homografting of tomato showed a much higher success rate (ca. 70%) than that of Brassicaceae, which occurred in the range of 19–34% (Table 2). However in heterografting where *Arabidopsis* was used as a scion on top of cabbage, radish, and tomato, water conduction never occurred (Table 2).

Table 2 Ratio of Successful Water Conduction Between Homografting and Heterografting of *Arabidopsis*, Members of Brassicaceae, and Tomato

Scions/Rootstock	Number of grafts	Successful water conduction (%)
Arabidopsis/*Arabidopsis*	36	19
Brassica/*Brassica*	12	29
Raphanus/*Raphanus*	12	34
Tomato/tomato	41	70
Arabidopsis/*Brassica*	10	0
Arabidopsis/*Raphanus*	11	0
Arabidopsis/tomato	28	0

Successful water conduction was considered when acid fuchsin dye was observed in the scions 17–25 days after grafting

Discussion

Grafting, which is a practice traditionally used to clone desired genotypes of woody plants, has become common in some annual field crops (herbs) because of the superior resistance of grafted combinations against pathogens and abiotic stresses. Grafting is usually carried out between different genotypes within a species, between different species within a genus, or between different genera within a family. Although graft incompatibility increases with genetic distance, it is not clear why any vascular plant cannot be grafted onto any other vascular plant. Little is known about the fundamental nature of the barriers that limit grafting. Obviously, there would be great potential agricultural benefits if it were possible to surmount these barriers and extend the range of graft compatibility. Creating desirable combinations of shoot and root phenotypes without grafting is not practical for traditional breeding and is a difficult task for genetic engineering when many genes are involved and organ-specific expression is desired. Grafting may enable the use of such combinations by bypassing these genetic constraints.

Homografting in *Arabidopsis thaliana* and in Other Species

Graft formation in *Arabidopsis* and in other plants is a complex process. In the present study we carried out several preliminary sets of homografting experiments using the inflorescence stems of *A. thaliana* to examine the process of grafting. During these preliminary experiments, we defined the best grafting method, the optimal grafting location within the inflorescence stems, and the appropriate timing for grafting. We showed that homografting *Arabidopsis* inflorescence stems involves several stages at the structural level: (1) an immediate wound response in both the scion and the rootstalk; (2) formation of a necrotic layer, which may disappear when cell division and callus formation fuse the scion and the rootstalk; (3) callus formation; (4) establishment of a regenerative wound cambium or regenerative vascular bridges; (5) establishment of intimate contact where the scion and the rootstalk can no longer be separated; and (6) establishment of a new functional vascular tissue connecting both partners. These processes take 15–21 days from grafting.

We observed similar processes with a similar time frame for tomato, cabbage, and radish grafting (data not shown). A similar time frame for graft formation was shown by others for tomato (Gebhardt and Goldbach 1988). The transport experiments with dyed water gave basically similar results. The fact that a much lower proportion of dye transport than that indicated by histologic examination was found in *Arabidopsis* homografts seems to indicate the formation of vessel ending that restricted dye transport, a known wound response (Indig and Aloni 1989).

Most molecular events associated with grafting are unknown but wound responses are inevitable during grafting. They have been studied in herbaceous plants, dealing with the regulation of transcription (Reymond and others 2000; Perez-Amador and others 2002; Strassner and others 2002). Several distinct signaling pathways respond to wounding, including pathways involved in the defense against biotic agents (for example, Fluhr 2001; Rowland and Jones 2001). Wounding elicits the activity of jasmonic acid, ethylene, nitric oxide, salicylic acid, and endogenous oligosaccharides, both locally and systemically (Rojo and others 1999; Walling 2000; Orozco-Cárdenas and Ryan 2002; Delessert and others 2004; Howe 2004; Schilmiller and Howe 2005). Some of the many genes involved in these responses may be specifically involved in graft formation, but no effort has yet been made to identify them or their role in grafting.

The wound response of the inflorescence stems of *Arabidopsis* has been described in detail at the developmental level (Flaishman and others 2003). In the first day after wounding, the wounded tissues show no structural response. After 6 days, regenerative vessel members differentiate from cortical parenchyma in a basipetal pattern, forming a vascular bypass around the wound. The process of forming regenerative vessel members peaks after 12 days. After 16 days the pith parenchyma loosens, suggesting senescence (Flaishman and others 2003).

An early, hallmark event in successful grafting is the production of callus tissue, which fills the space between the scion and the rootstalk (Jeffree and Yeoman 1983). In the course of callus formation, cells from the cut surface quickly increase in size and divide. Once the two components of the graft (the scion and the rootstock) are in intimate contact, cell divisions give rise to parenchyma, which interlocks and connects the scion and the rootstock. Yeoman and others (1978) found that in tomato grafts, cell death in the grafted area forms a necrotic layer. In Solanaceae, this necrotic layer was produced in both compatible and incompatible grafts (Yeoman and others 1978). Interface callus is formed from undamaged cells, and the development of future vascular connections may depend on this cell-to-cell contact.

The establishment of successful grafts requires the redifferentiation of parenchyma into new xylem and phloem or the formation of a regenerative cambium. In homografts of *Arabidopsis* and tomato, the differentiation of callus parenchyma into new vascular connections is completed after 15 days. Turquois and Malone (1996) found that hydraulic connections in the graft union in tomato became functional 5 days after grafting, probably before complete differentiation of vessel members and

when only the parenchymatic bridge was intact. These researchers showed a successful graft union with several functional phloem and xylem connections that cross the graft interface after only 15 days.

Grafting Incompatibility

In cases of graft incompatibility, the stock and scion fail to form a successful graft. In this study we identified partial graft incompatibility when *Arabidopsis* was grafted on stocks of cabbage and radish. Such partial incompatibility resembles the localized incompatibility in trees that is associated with malformation of graft unions, resulting in mechanical weakness and subsequent breakdown of that union (Errea 1998). A stronger level of graft incompatibility was observed when *Arabidopsis* was grafted onto tomato rootstock. In this case, the necrotic layer that developed in the tomato plants seemed to inhibit the differentiation of vascular tissue in the *Arabidopsis* scion, either directly or indirectly, and thus prevented full vascular graft union formation between the two plants. Despite the lack of vascular connections, *Arabidopsis* scions were able to flower and develop seeds when grafted onto tomato plants. This may indicate some level of nonvascular functional connections between the two plants, probably of parenchyma cells, and further emphasizes the usefulness of *Arabidopsis* as a model plant for the study of grafting biology.

Grafting has significant economic implications. However, its regulation and the genetic and cellular factors involved in this process remain largly unknown. Therefore, we do not know if grafting represents a direct response to a stimulus, to changes in hormonal signaling (especially auxin), or to a combination of these two factors. Here we showed that *A. thaliana* can be used to study various levels of grafting incompatibility. Although not a tree, *A. thaliana* may provide valuable insights into the genetic control of graft formation by allowing the identification of many genes and cellular processes involved in grafting.

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