

14 Grafting and Rootstocks

Individual plants of many horticultural crops are multiple genetic specimens. Rather than consisting of one single genotype, they are two or more distinct, different genotypes joined together as a single plant. The multiple components are identified as: (i) the rootstock; (ii) the interstem; and (iii) the scion. Grafting (which includes budding) is the process of combining these components in such a way as to establish vascular continuity between them to produce a composite genetic organism that grows as a single plant. Grafting may be natural or actively accomplished by humans. Most of this chapter will deal with forced grafting with only a brief discussion of natural grafting.

The rootstock, stock, or understock is that component of the plant that fuses with the scion and provides the plant's root system. Stock is synonymous with both rootstock and understock, however, understock implies that the lower portion of the plant provides both the root system and some of the trunk. Rootstock implies that only the root system is provided by the lower piece. Rootstocks are chosen for many reasons including soil adaptability, dwarfing, pest resistance, precocity and many others. In some cases when grafting is performed high on the trunk of the rootstock, the rootstock may also provide the trunk and even scaffold limbs in some cases to the finished plant.

The scion produces the plant's shoot system and is the component that produces the desired commodity in most cases, which are usually flowers or fruit. In perennials, the scion is almost always vegetatively propagated. In grafted vegetables, the scion is usually propagated via seed.

An interstem is the third genetic component of some grafted plants and is often selected to provide compatibility between the rootstock and the scion. It may also impart desired characteristics to the scion or rootstock.

Grafting is the general term given to the process of combining dissimilar genotypes into one plant.

Budding is a form of grafting in which a single vegetative bud is used as the scion or interstem. Grafting often implies that one or more buds on a common stem piece are combined with the rootstock or interstem.

Perennial ornamental and fruit crops are the standard grafted crops that are familiar to most horticulturists. Several annual vegetable crops are increasingly being grown as grafted plants and interest in using them in commercial production is increasing rapidly.

A good rootstock should possess as many of the following characteristics as possible that are appropriate for the crop: (i) is affordable; (ii) has long-term graft compatibility; (iii) is easily propagated; (iv) promotes precocity and productivity; (v) controls scion vigor; (vi) conveys pest resistance; (vii) improves stress tolerance; and (viii) has minimal suckering.

It is far beyond the scope of this text to cover all crops that may be grafted and their rootstocks. A general discussion of grafting and rootstocks follows with specific references to commonly grafted species.

Reasons for Grafting

There are many reasons for grafting (Fig. 14.1). The main reason for grafting is the clonal propagation of cultivars for commercial production. The story behind the development of apple rootstocks is a good illustration of why grafting developed and how horticultural technology often initially addresses one problem then expands in many directions to enhance production in many ways.

Apple (*Malus* spp.) is difficult to propagate via cuttings and does not come true from seed. To facilitate the propagation of scions desired for their fruit quality and productivity, grafting (and budding) were developed. In propagating desired scions by grafting, fruit growers soon realized that grafted trees came into production much sooner

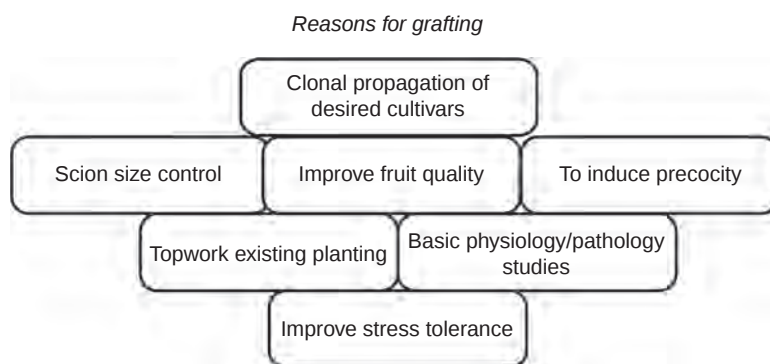


Fig. 14.1. Reasons for grafting.

than seedling trees. Seedlings often took 15–20 years to begin producing a full crop, grafted trees reached full productivity in as few as 5 years. In addition, some grafted trees tended to be smaller than seedling trees. Smaller trees are easier to manage and more of them can fit onto a unit of land. Land use efficiency was increased in both space and time. From the need to efficiently propagate desired scions, and entire industry was changed.

The ‘original’ apple rootstock, ‘Paradise’, was selected in the 16th century. The ‘French Paradise’ rootstock induced precocity and caused scion dwarfing. The ‘English Paradise’ or ‘Doucine’ rootstock was less dwarfing. Over time, these rootstocks were mixed, mislabeled and became part of a mixture of genotypes collectively called ‘Paradise’. By the 1800s, 14 different strains of ‘Paradise’ rootstocks existed (Rivers, 1865).

To clear up the confusion surrounding the different strains of ‘Paradise’, an English researcher at the East Malling fruit research center in Kent, England reclassified samples (29 from Britain, three from France, one from Germany and one from The Netherlands) using botanical and anatomical comparisons. Each stock was evaluated for size control, propagation, and productivity. Eventually 27 rootstocks were released with the East Malling designation, originally EM.1–27 which was changed to M.1–27. Rootstocks were also classified according to their effect on scion vigor and were renamed to remove the confusion surrounding the clones. Rootstocks were classified as: (i) dwarfing; (ii) semi-dwarfing; (iii) vigorous; and (iv) very vigorous (Hatton, 1917). Crosses were made between M rootstocks and the ‘Northern Spy’ apple at the John Innes Institute in Merton

England to introduce resistance to the woolly apple aphid (*Eriosoma lanigerum*) to produce the M.M. series of apple rootstocks, Malling-Merton 101 through to 115 (Jackson, 2003).

Thus grafting originated to obviate problems surrounding the inability to propagate desired varieties of plants directly from seed or obtain clonal plants via cuttings. Over time many other benefits of grafted plants were observed and are now noted as reasons for grafting.

Low success rates or prohibitive costs associated with cuttings, tissue culture or seed propagation may warrant grafting. Grafting to avoid an unfruitful juvenile period is a major catalyst for grafting fruit and nut species. Topworking, a specific form of grafting, is used to change the scion cultivar of an established planting when market demands or production trends suggest a change would be prudent. Pollinizers can also be grafted into established trees to improve fruit set. Injuries can often be repaired by grafting. Disease indexing of stock plants and studies of basic plant physiology, especially long-distance signal transmission in plants, both utilize grafting.

Production Traits Affected by Grafting

There are a number of production traits that may be affected by grafting. These include: (i) improved biotic and abiotic stress resistance; (ii) size control; (iii) induction of precocity; (iv) influence on time of bloom; (v) accelerating plant growth rate thereby reducing nursery production time; (vi) obtaining special plant forms such as weeping cherries or tree roses; and (vii) improved fruit and nut quality.

Size control

Many rootstocks influence scion growth and ultimate canopy size. Larger trees tend to have a larger root system and the dwarfing effect of different rootstocks may (Fernandez *et al.*, 1995) or may not (Atkinson *et al.*, 1999) be related to the size of their root system. The mechanism for dwarfing is probably very complex and no single unified theory for how it is accomplished has yet been developed.

Many studies on the vascular systems of grafted plants have been performed in the hope of elucidating some of the mechanisms for rootstock action, particularly on size control of the scion (Beakbane and Thompson, 1947; Simons, 1986; Misirli *et al.*, 1996). There is good evidence that there are measurable differences in the vascular anatomy of different rootstocks, particularly in the xylem, which may reduce water movement to the scion and thereby dwarf it (Beakbane and Thompson, 1947; Simons, 1986). In addition, root systems that dwarf the scion have a lower xylem to phloem ratio than root systems that do not dwarf the scion (Beakbane and Thompson, 1947). Rootstocks which are not dwarfing also have larger vessel elements than those that are dwarfing.

Dwarfing rootstocks reduce the hydraulic conductivity of the stem xylem (Atkinson *et al.*, 2003) and dwarfing rootstocks have increased resistance to water movement at the graft union itself than non-dwarfing rootstocks. These differences in conductivity due to differences in vascular anatomy may be related to limited auxin transport across the graft union (Soumelidou *et al.*, 1994). Auxin plays a vital role in the redifferentiation of xylem from callus that forms at the graft union (Hess and Sachs, 1972; Parkinson and Yeoman, 1982; Weatherhead and Barnett, 1986; Savidge, 1988; Ugglä *et al.*, 1996) and any aberration in auxin levels would certainly alter the redifferentiation process. Signals from the scion, particularly auxin, move more slowly in dwarfing rootstocks compared with non-dwarfing rootstocks (Kamboj *et al.*, 1997) even though auxin moves mostly in the phloem, and there is more phloem in scions on dwarfing stocks compared with non-dwarfing stocks (Beakbane and Thompson, 1947).

In many scion-rootstock combinations, scion growth is reduced relative to non-grafted plants and the efficiency in which dry matter can be partitioned into fruit is increased with more dry matter going into the fruit and less going into vegetative

growth. This attribute is called yield efficiency and is measured as the weight of fruit produced per unit of trunk cross-sectional area measured at 30 cm above the orchard floor. Different scions on the same rootstock often have different yield efficiencies.

Two key attributes of any rootstock are:

1. What is the degree of dwarfing compared with a standard seedling tree?
2. What is the increase in yield efficiency?

Both attributes combine to establish the appropriate planting density for each scion-rootstock combination. If plants are spaced too closely yield may decline due to interplant competition. If plants are too far apart, they won't fill their allotted space and productivity per unit land area suffers.

Reduced scion growth makes plant management easier. All production operations (pruning, thinning, harvesting) can be performed from the ground without introducing ladders into the field. It takes time to walk up and down ladders and to move them from tree to tree. Ladders also increase the chances for accidents in the field. Smaller trees also require less pruning. Smaller, more compact canopies make foliar sprays of pesticides, growth regulators or fertilizers easier to apply and require less material per application.

Induction of precocity

Most scion budding or grafting stock is mature tissue and has outgrown the juvenile stage in which flowering is lacking. Even so, many rootstocks are known to induce flowering and fruiting in many scion cultivars whether the tissue is juvenile or not. Early and consistent flowering is desirable to bring a commercial operation into production as early as possible thereby making it more sustainable.

Fruit quality control

Fruit quality is a matter of consumer preference. Specific measurements can be made on chemical composition and physical characteristics to quantify quality attributes. The quality of deciduous fruit crops is affected by the rootstock due to rootstock effects on growth characteristics directly related to crop load and canopy management (fruit size, firmness, skin color) rather than direct effects of the rootstock on specific chemical quality parameters (Castle, 1995). On the other hand, in semi-tropical and tropical crops such as citrus (*Citrus* spp.) and

avocado (*Persea americana*), the rootstock can affect fruit quality by directly affecting chemical components of quality. These components of quality are largely a function of water relations within the plant that are affected by the rootstock.

Topworking

Grafting is often used to change the cultivar in an established planting without having to replant. Topworking consists of removing the established cultivar through severe pruning and establishing the new cultivar via grafts onto the stubs left by pruning. Another variation to topworking is the incorporation of a pollinizing cultivar intermittently throughout an orchard, rather than planting entire trees of the pollinizer.

Stress tolerance

The rootstock can influence a plant's tolerance to many forms of stress encountered during production. The tolerance may be a direct tolerance of the rootstock to the stress or an alteration of a scion characteristic by the rootstock that makes the scion more tolerant of the stress.

A rootstock's tolerance to the soil in which it grows is important. Rootstocks vary in their ability to anchor the plant in the soil and thus many crops require support in the form of a trellis or individual stakes. Tree anchorage is often related to brittleness of roots and depth of rooting. For example, trees on the apple rootstock M.9 are very poorly anchored due to brittle roots (Webster, 2002) probably caused by short fibers and a high proportion of root bark to xylem (Ferree and Carlson, 1987). To improve anchorage, scions are often budded higher on the rootstock so that the tree can be planted deeper, allowing root development along the rootstock shank. Rootstocks also differ in their tolerance of chemical properties of the soil such as pH, salinity, sodicity, or nutrient excesses or deficiencies.

Physical soil properties such as texture and water status influence productivity. Some rootstocks tolerate fine or coarse soils and wet or dry soils better than other rootstocks. Rootstocks are often utilized for their ability to impart tolerance of drought and flooding conditions. As water rights becomes a greater issue in agricultural production and climate change impacts water availability, the importance of rootstock choice may become even greater. While dry soil conditions are often overcome with irrigation,

flooded soil conditions are often much harder to rectify. Rootstock influence on a plant's ability to tolerate waterlogged soil is especially important in crops that are intolerant of such conditions (*Prunus*) and less important for tolerant crops (*Vitis*). The mechanism by which certain rootstocks impart tolerance to flooded soil conditions seems to be associated with their ability to form adventitious roots (Anderson *et al.*, 1984). Adventitious roots have extensive aerenchyma tissue which would allow oxygen to enter the rhizosphere during flooding (Al-Husainy and Jackson, 2001).

There are many soil-borne pests that negatively impact plant growth. One of the most widely observed effects of rootstocks is their resistance to specific pests. Many of these diseases have particular economic importance to the fruit industry. They include: (i) bacteria, e.g. fire blight (*Erwinia amylovora*); (ii) fungi, e.g. crown rot (*Phytophthora cactorum*); (iii) insects, e.g. woolly apple aphid (*Erisoma lanigerum* Hausm.) and grape phylloxera (*Daktulosphaira vitifoliae*); (iv) viruses (citrus tristeza virus in citrus); (v) and nematodes (*Meloidogyne* spp. on peaches and walnuts). The resistance of the rootstock to the pest imparts increased productivity to the scion by virtue of the fact that the rootstock functions more efficiently. In addition, peach rootstocks are chosen for their resistance to the orchard replant syndrome, a malady that prevents the successful re-establishment of peaches on land previously planted with peaches. The mechanism for this resistance and resistances imparted to the scion are poorly understood.

Winter injury to both rootstock and scion are important in colder regions. Rootstocks vary in their hardiness from not hardy to extremely hardy, usually depending on their region of development and adaptation (Quamme, 1990; Quamme and Brownlee, 1997). The rootstock can also influence the hardiness of the grafted scion (Rollins *et al.*, 1962; Durner and Rooney, 1988; Durner, 1990a). The mechanisms for such differences in hardiness may be related to the rate of scion maturity in the fall or regrowth in the spring (Ferree and Carlson, 1987). Early-blooming scion cultivars are undesirable in regions where late spring frosts regularly occur. A late frost can totally eliminate a crop. Rootstocks can influence the time of scion bloom in many fruit crops (Durner, 1990a). Even several days delay in bloom to avoid frost or freeze injury can mean the difference between little or no damage and complete crop failure.

Plant physiology/virology investigations

Many horticulturally useful plants are hosts to viruses that may significantly decrease the vigor or productivity of the plant, but otherwise may be present in the plant asymptotically. If a sample of the suspect plant is grafted onto a sensitive relative that is susceptible and readily shows symptoms of the virus, the virus will be transmitted across the graft union and the presence of the virus can be verified. This procedure is called virus indexing. It is used less frequently now with the availability of specific enzyme-linked immunosorbant assays (ELISA) or assays based on the polymerase chain reaction (PCR).

Poinsettias (*Euphorbia*) are induced to branch by grafting a scion onto the desired cultivar to transmit a phytoplasma (cell-wall-less bacterium) which induces the desired branching (Mudge *et al.*, 2009).

In apples, virus infection of rootstocks may reduce scion vigor (which is often desired) as compared with scions grafted onto virus-free rootstocks. For example, scions on virus-free EMLA.9 rootstock are more vigorous than those on virus infected M.9 clones (Autio *et al.*, 2001).

Grafting has been used extensively in basic plant physiology studies, particularly to study the translocation of signaling molecules and nutrients within the plant. Some examples include: (i) the translocation of secondary metabolites (Wilson, 1952); (ii) the flowering stimulus (Zeevaart, 2006); (iii) the potato tuberization stimulus (Ewing and Struik, 1992); (iv) plant hormones (Foo *et al.*, 2007); and (v) RNAs (Tournier *et al.*, 2006).

Rootstock Propagation

The ease of propagation was once the criteria for selection of many rootstocks. As more reliable means of propagation were introduced (Fig. 14.2), criteria for selection became more diverse to include vigor control, pest resistance, adaptability to soils and climate, etc.

For much of history, rootstocks were propagated from seeds collected from wild indigenous populations and scion performance on these seedlings was extremely variable. In the 17th century, clonal propagation of selected seedlings began (Webster, 1995). The first propagules were probably suckers arising from the base of superior trees. Eventually, methods of layering and stooling became popular and only rootstocks that could be easily propagated with these methods were selected. As selection criteria shifted from ease of propagation to greater adaptability and sustainability, rootstocks have been selected that are often not easy to propagate.

Seeds

Most rootstocks grown throughout the world are propagated by seed (Webster, 1995). Many fruit crop rootstocks including peaches, nectarines (*Prunus persica* L.), apricots (*Prunus armeniaca* L.), Asian pears (*Pyrus pyrifolia* (Nakai)), and *Citrus* species are propagated by seed. While seed propagation is easier and more economical for the nurseryman, seed-propagated rootstocks are usually far less desirable for the grower since seedling rootstocks are often inferior to clonally propagated

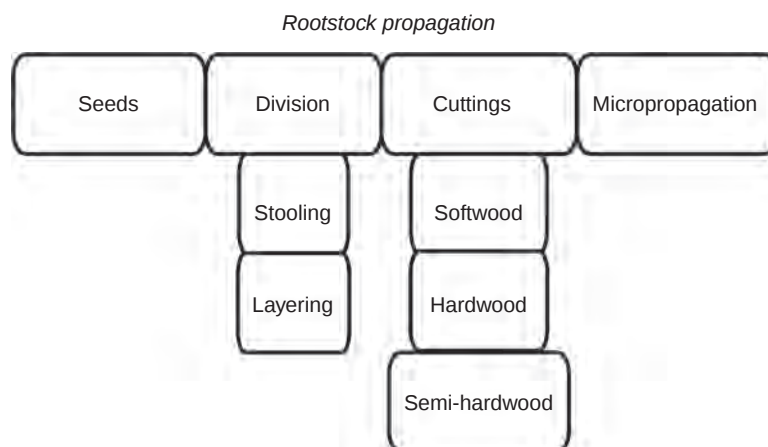


Fig. 14.2. Common methods of rootstock propagation.

ones. However, in species where viruses are not transmitted through seeds, and virus-infected clonal material is evident, seedlings are probably more beneficial. Seedlings also help avoid transmission of root-borne diseases during clonal propagation.

The largest drawback of using seedlings as rootstocks (besides the obvious lack of desirable traits from specific clonal rootstocks) is the lack of uniformity among seedling rootstocks. Uniformity among seedling rootstocks can be improved by using seed of a specific clone or from a self-fertile cultivar well isolated from other cultivars or by using seeds from apomictic rootstocks. Apomictic seeds are widely used in the citrus industry. Apomictic seeds are formed when a cell in the embryo sac fails to undergo meiosis (reduction division) and forms an embryo genetically identical to the seed-bearing plant. Thus apomictic seeds are an asexually derived, clonal seed propagule.

Division

Propagation by division has an advantage over cuttings in that propagules are not removed from the parent plant until after roots are well established on the propagule. The main forms of division used in rootstock propagation are: (i) stooling; and (ii) layering.

Stooling

Rootstock plants that are at least 1 year old in the rootstock nursery are cut, removing most of the above-ground portion of the plant. Shoots arise from buds on the remaining stump. Soil or sawdust is used to mound the bases of these shoots to blanch them and induce rooting. This mounding process is performed several times during the summer until 15 cm of the shoot is covered. Temperature, moisture and adequate oxygen must be maintained around the rootstock stem to ensure success. Pest control is also important. After leaf fall, the mounding material is removed and the rooted stems are removed from the stump to be placed in the budding nursery where the appropriate scion cultivar will be grafted to it. The stump left behind is used again the following season for stooling.

Layering

Layering is similar to stooling except that not all rooted shoots are removed from the parent plant at

harvest. Several shoots are left on the parent plant and in the spring, they are bent to horizontal before bud break and held to the soil with pins, then covered with a shallow 3–4 cm layer of soil. Axillary and adventitious buds will grow and their bases will be etiolated since they are soil covered. Rooting will occur in this etiolated zone. Mounding and harvesting are performed as in stooling.

Cuttings

The three main types of cuttings used in rootstock propagation are: (i) softwood (summer) cuttings; (ii) hardwood (winter) cuttings; and (iii) micropropagation. Micropropagation is used mainly for hard-to-root species or for rapid multiplication of rootstocks that are in short supply. Two other forms of cutting occasionally used in rootstock propagation are semi-hardwood cuttings and root cuttings.

In any form of cutting, the parent from which the cutting is taken must be healthy and at the right stage of development. Once the cutting is taken, it may require further wounding or treatment with auxin to stimulate root initiation. A major factor that makes one rootstock easy to propagate and another one extremely difficult to propagate is related to the amount of auxin in the cutting shoot base (Alvarez *et al.*, 1989). Difficult-to-root species often have high levels of bound, inactivated indole acetic acid (IAA) in them compared with easier-to-root species. Free auxin, which is at a maximum 1 day after cutting, leads to dedifferentiation of parenchyma cells near vascular bundles in the base of cuttings. These dedifferentiated cells then form root primordia about 100 h after cutting. The final consideration for successful rooting is the placement of cuttings in an environment to encourage rooting.

Softwood cuttings

Softwood cuttings are taken from parent plants early in the growing season when tissues are soft and succulent or just beginning to lignify. They are generally 5–20 cm in length and are removed from the parent just below a node. Leaves are removed from the bottom third of the cutting and the remaining leaves may be trimmed to half their size. Cutting bases are often treated with auxin to stimulate rooting and a fungicide to prevent rot if it is known to be a problem. Cuttings are placed in a moist but well-drained rooting medium usually in a partially shaded greenhouse or lath house with

high humidity maintained with intermittent misting or fogging. Misting or fogging is decreased as rooting progresses and plants are often ready to be transplanted in 4–6 weeks.

Hardwood cuttings

Hardwood cuttings are taken from parent plants either after leaf fall or before bud break during the dormant season. Cuttings taken during the middle of the dormant season usually do not root well. Cuttings taken from severely pruned plants are preferred to cuttings from any other source. Cutting bases are treated with auxin either as a long soak in a dilute (250–500 ppm) solution of auxin or a quick dip in a concentrated (2500–5000 ppm) solution. Auxin in talcum powder may also be used. In all cases, it is important for the cut base to be exposed to the auxin so that adequate uptake can occur.

Many hardwood cuttings benefit from wounding prior to auxin treatment. Wounding most often consists of cutting the base of the cutting longitudinally for several centimeters to expose the cambium, which allows more auxin to enter the cutting and stimulate rooting. Wounding increases both the number of cuttings that root and the number of roots per cutting.

After wounding and auxin treatment, cuttings are placed vertically with their bases inserted into 10–20 cm of rooting material such as sterile compost or sand and kept at 21°C or higher for 8–10 weeks. In most cases only the base of the cutting is heated, usually by heating cables in the medium. Keeping the entire cutting at 21°C or greater may improve rooting, but is often more costly. Cambial cells dedifferentiate into callus tissue which then differentiates into roots.

It is imperative to prevent excessive water loss from cuttings during the initial weeks following placement in the rooting medium. Excessive water loss leads to a dramatic decrease in rooting success. Additionally, once callusing and rooting begin, excess water can be equally detrimental. Once rooted, plants are transferred to the grafting nursery in the spring.

Micropropagation

Rootstocks are often propagated by micropropagation, especially hard-to-root species or those in short supply. There are many different recipes for micropropagation media, but they all consist of a

liquid or solid carrier such as agar, along with nutrients, minerals, sugars, vitamins, and hormones. Axillary shoot proliferation is the micropropagation method most usually employed for rootstock propagation. The distal 5 mm of an actively growing shoot are excised and cultured on an appropriate medium. The axillary buds contained in this shoot meristem begin to grow into shoots which are separated from the meristem. Each shoot is then ready to be similarly propagated after several weeks' growth. Using this method, large quantities of new plants can be generated without maintaining large quantities of stock plants. This method also avoids mutations into off-types that are often associated with other tissue culture methods that rely on dedifferentiation of tissue into callus followed by differentiation into shoots then roots via manipulation of hormone levels in the medium.

Once enough shoots are produced via axillary shoot proliferation, root formation is induced on the propagules by altering the growth regulators in the growing medium, usually high auxin and relatively low gibberellins and cytokinins. Once rooted, plants are slowly acclimated to 'normal' growing conditions.

Physiology of Grafting

The physiology of grafting has long fascinated horticulturists. Both the physiology of graft wound healing and the physiology of the effects grafting causes have been investigated for years, however, our knowledge of these mechanisms remains fairly incomplete (Atkinson and Else, 2001).

Basic biology of grafting

The biology of grafting starts with the formation of a graft union between the grafted parts. Union formation is particularly interesting since a number of events must occur in sequence for success. A wound is initiated by the grafting cut which leads to cellular dedifferentiation, cell division, followed by the establishment of a functioning vascular system connecting the two grafted pieces.

The first and most important step in establishing a successful graft union is lining up the cambial layers of the two grafted tissues. The cambium is one cell layer thick, thus the grafter must be meticulous in aligning these two layers during graft placement. Since a layer one cell thick is not easy to see, lining up the region where the cambium is

located is often sufficient for success. To ensure that the cambia remain aligned and maintain contact through the healing process, the two grafted pieces are bound together with rubber ties, twine or similar materials that eventually disintegrate.

The cuts made during grafting induce a wound response in both grafted pieces leading to cell dedifferentiation and callus formation. The scion side of the graft union is often more prolific than the rootstock or interstem side in terms of callus production. Callus from both sides of the graft union must commingle to form a successful callus bridge between the two parts.

The next crucial step is the formation of a vascular bridge between the two pieces via differentiation of cells in the callus that commingled during the wounding response. New cambial cells differentiate from parenchymatous callus cells. These new cambial cells give rise to xylem and phloem; xylem generally forms first followed by phloem. Following the formation of a vascular bridge between the grafted entities, a new vascular cambium must develop to produce secondary xylem and phloem (Hartmann *et al.*, 2002). Auxin released from vascular strands in the scion and rootstock stimulate the differentiation of vascular tissue in the wound-induced callus (Aloni, 1987; Mattsson *et al.*, 2003).

Many factors influence whether or not a graft union 'takes'. Genotype has a large influence on grafting success, some species are easier to graft than others. In addition, the genotypes of the scion/interstem/rootstock are important in that the closer they match, the more likely there will be a successful union.

Temperature can effect cambial growth and callus formation. In hardwood grafts, plants are often held at temperatures between 4.5 and 5.0°C for several months to encourage callus formation and wound healing without stimulating vegetative growth of the scion. Cooler temperatures inhibit callus growth and wound healing and higher temperatures may lead to excessive callus growth and depletion of carbohydrate reserves within the plant. Moderate temperatures are also required in other types of grafting to promote cambial growth and wound healing.

Moisture must be maintained around the graft to prevent desiccation. Grafts are often wrapped with various materials or covered with wax to prevent desiccation. In some types of grafting, the rootstock must be actively growing. T-budding, chip budding and bark grafting all require that the bark is slipping, which is the result of active cambial growth.

The craftsmanship of the grafter is extremely important. Some people are meticulous and are very good at aligning cambial tissues to promote the successful graft union while others are not. Cambial alignment is paramount to grafting success.

Graft incompatibility

When a graft union fails to occur or when a graft union fails to hold after apparent success, the graft is said to be incompatible. Sometimes incompatibility is immediate and the union never occurs. In many cases, the graft appears successful but fails several months to many years after 'taking'. Compatibility should be considered on a continuum from totally compatible to totally incompatible. Some unions which appear incompatible may actually be productive if proper horticultural practices such as training and trellising are used.

A major cause of immediate incompatibility is the failure to make vascular connections between the two grafted pieces, often due to misalignment of the cambia. Aligning grafting cambia is as much an art as it is a science. Sometimes the vascular connection does not occur due to adverse environmental conditions during the healing process. Usually insufficient water supply to the scion or unacceptable temperatures prevent the formation of a vascular connection. Disease can also play a role in causing immediate incompatibility, thus it is important to use only clean stock.

Genetic incompatibility occurs when there is some form of cellular miscommunication between the two grafted pieces, preventing the successful establishment of a union. Many theories have been proposed as the mechanism for genetic incompatibility, however, none of them really target the cause or explain why the union failed but rather describe the symptoms and what happens.

Some symptoms of incompatibility besides the obvious lack of a physical union between the two pieces include: (i) yellowing of foliage after leaf expansion in the spring; (ii) declining vegetative growth over time for no other apparent reason such as disease or poor fertility; or (iii) a clean break of the scion from the rootstock at the grafting site long after the graft appeared to be successful.

In general grafting is most successful with dicots and gymnosperms but some limited success has been achieved with monocots. The more closely related the scion and the rootstock are, the more likely there is to be a successful graft union.

Scion–rootstock hormonal interactions

The interactions observed among rootstocks, interstems and scions are often attributed to alterations in hormone levels throughout the plant. In addition, whether or not the graft is compatible is often attributed to hormonal signaling among the grafted segments.

Auxin is produced in shoot apices and cytokinins are produced in the roots. Decreased auxin transport to the roots leads to an increase in the synthesis and export of cytokinin from the roots to the shoots (Bangerth, 1994). An increase in cytokinins in the xylem sap leads to an increase in auxin synthesis and translocation from the shoots which in turn would decrease cytokinin production by the roots. The balance of auxin and cytokinin between shoots and roots leads to the normally observed balance of growth between the root and shoot (Sorce *et al.*, 2002). When the normal balance is altered, the growth observed is altered. One theory is that invigorating rootstocks do so by increasing the amount of cytokinins exported from the roots to the shoots resulting in more robust scion growth (Sorce *et al.*, 2002). Dwarfing rootstocks may reduce the basipetal transport of auxin to the roots which reduces the amount of root-produced cytokinin and gibberellin translocated to the shoot, resulting in reduced shoot growth (Van Hooijkonk *et al.*, 2010). Greater cytokinin concentration in apple xylem is associated with vigor-inducing rootstocks (Kamboj *et al.*, 1999).

Gene transcripts associated with auxin signaling have been shown to move from pumpkin (*Cucurbita maxima* Duch.) rootstocks to melon (*Cucumis melo* L.) scions (Omid *et al.*, 2007) providing evidence for signal movement from the rootstock to the scion. Squash (*Cucurbita* spp.) xylem sap contains significant amounts of cytokinin that can inhibit auxin-induced cucumber hypocotyl elongation, suggesting that cytokinins translocated from the roots may alter scion growth by affecting the response to auxin (Kuroha *et al.*, 2005).

Incompatibility in cucurbit grafting has been shown to occur at about 25 days after grafting and is attributed to a hormonal imbalance, particularly of auxin and ethylene in the rootstock (Aloni *et al.*, 2008). The source of the incompatibility seems to be extreme sensitivity to auxin in the rootstock. Auxin produced in the scion is translocated to the rootstock once the graft connection is made and when it reaches some threshold level in the roots, it

triggers inhibited root growth and enhanced root decay. Incompatible rootstocks are either more sensitive to auxin or accumulate more auxin than compatible ones. The degradation induced by auxin may be due to damage from oxidative stress caused by ROS build up, either due to increased production or reduced removal via peroxidases and other enzymes, in incompatible root systems.

High root auxin levels may trigger ethylene production in incompatible rootstocks leading to their degradation (Mulkey *et al.*, 1982; Rahman *et al.*, 2001). Roots produce increased amounts of ethylene following auxin treatment and incompatible rootstocks produce more ethylene than compatible ones (Aloni *et al.*, 2008).

There are other observations illustrating the involvement of hormone signaling in rootstock–scion interactions. Cotton (*Gossypium* spp.) cultivars vary in their time of leaf senescence, some senesce early while others senesce late. When early senescing scions are grafted onto late senescing rootstocks, scion leaf senescence is significantly delayed. When late senescing scions are grafted to early senescing rootstocks, scion leaf senescence is accelerated. Late senescing rootstocks produce cytokinins which are translocated to the scion to delay leaf senescence (Dong *et al.*, 2008).

Salt tolerance in potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*) can be induced by grafting salt-intolerant scions onto salt-resistant rootstocks (Chen *et al.*, 2003; Dodd, 2007; Etehadnia *et al.*, 2008; Albacete *et al.*, 2009). The salt tolerance is conferred by enhanced ABA production and subsequent translocation to the scion by the salt-tolerant rootstock.

Watermelon (*Citrullus lanatus*) grafted to squash (*Cucurbita* spp.) roots are more vigorous than non-grafted plants due to enhanced cytokinin production and translocation from the rootstock to the scion (Yamasaki *et al.*, 1994). Sweet cherry (*Prunus avium*) productivity on different rootstocks was shown to be positively related to higher levels of cytokinins in scion xylem extracts (Stevens and Westwood, 1984). Higher levels of cytokinin enhance tissue sink strength (Mothes and Engelbrecht, 1961) which may help explain the effect of rootstock on scion vigor and productivity.

Grafting Ornamentals

One of the major problems in identifying problems in ornamental plants is that many species are grafted

and the rootstock is often unknown and is often not considered when trying to diagnose problems (Ball, 2004). It is important to keep good records of ornamental specimens so that in later years, problem diagnosis may be simplified by knowing both the scion and the rootstock. Incompatibility is a serious problem with oak (*Quercus* spp.), maples (*Acer* spp.), yews (*Taxus* spp.), and Douglas fir (*Pseudotsuga menziesii*) in that it is often manifest 4 or more years after planting. This 'delayed' incompatibility has essentially wasted years in developing the desired landscape. A solution is to utilize specimens that are on their own roots.

Even in compatible combinations problems between the scion and the rootstock can occur. Often the rootstock will send up a vigorous sucker that if not removed promptly will outcompete the scion and result in an undesirable plant. For example, crab apples (*Malus* spp.) are often grafted onto apple (*Malus domestica*) seedlings with such results. Root suckers must be removed promptly.

In a number of ornamental species, the rootstock does not end 5–10 cm above the ground, but rather may extend as the trunk of the tree 1–1.5 m. Weeping forms of trees such as tabletop elm (*Ulmus glabra* 'Pendula') and weeping mulberry (*Morus alba* 'Pendula'), are budded to rootstocks about 1.5–2 m above ground (Ball, 2004). The trunks of these trees must be kept free of shoots.

Vegetable Grafting

Vegetable grafting has been practiced for many years in Eastern Asia to alleviate soil-borne pest problems and to enhance plant vigor and productivity under adverse environmental conditions. Grafting vegetables has become a very popular pest management technique worldwide and continues to grow in popularity.

In Japan and Korea, the majority of muskmelons (*Cucumis melo*), watermelons (*C. lanatus*), cucumbers (*Cucumis sativus*), tomatoes (*S. lycopersicum*), and eggplants (*Solanum melongena*) in both field and greenhouse production utilize grafted transplants (University of Arizona, 2012). Nearly all of the watermelon production in the Almeria region of Spain utilizes grafted plants to control Fusarium wilt. Most grafted vegetables in North America are tomatoes used for greenhouse production; however, grafting heirloom cultivars onto suitable rootstocks for organic production is becoming increasingly popular. As the use of the fumigant

methyl bromide is phased out, interest in grafted vegetable transplants is likely to increase.

The vegetable crops most frequently grafted are tomatoes (*S. lycopersicum*), cucumbers (*C. sativus*), muskmelons (*C. melo*), watermelons (*C. lanatus*), eggplant (*S. melongena*), and peppers (*Capsicum annuum*). Before embarking on a fully fledged switch to grafted plants, growers are encouraged to experiment on a small scale and consult with local experts. The major benefits of grafted vegetable transplants include: (i) resistance to soil-borne pests; (ii) tolerance to abiotic stresses such as chilling, heat, and salt stress; and (iii) increased plant vigor and yield. While robotic grafting machines are available, grafting by hand is practiced worldwide.

Tomato grafting

Tomato rootstocks are selected for resistance or tolerance to Fusarium wilt, bacterial wilt (*Ralstonia solanacearum*), Verticillium wilt, tomato corky root (*Pyrenochaeta lycopersici*) and root knot nematodes (*Meloidogyne* spp.). These rootstocks are often hybrids of cultivated tomato cultivars (*S. lycopersicum*) to create intraspecific hybrids or between cultivated tomatoes and wild relatives to create interspecific hybrids. Interspecific hybrids may suffer from lack of germination uniformity, but are often more robust compared with intraspecific hybrids.

Rootstock and scion vigor should also be considered in their selection. A less vigorous scion is expected to become more vigorous if grafted to a vigorous rootstock. The rootstock should not be so vigorous as to cause excessive vegetative growth of the scion which might result in reduced yields.

When dealing with cultivars and rootstocks with resistance to tomato mosaic virus (ToMV), the level of resistance of both the scion and the rootstock must be considered. The scion must be as resistant as the rootstock, or more resistant than the rootstock to prevent a hypersensitive reaction from occurring at the graft union should the scion become infected with the virus. A hypersensitive reaction induces cell death which would lead to a sudden collapse of the graft union, sudden plant death, and total crop failure. Many seed companies classify resistance as tm-1 or tm-2, with tm-2 being more resistant.

Tomatoes are most commonly grafted using the 'Japanese Grafting Method' also called tube grafting (Fig. 14.3). In this method, scion and rootstock

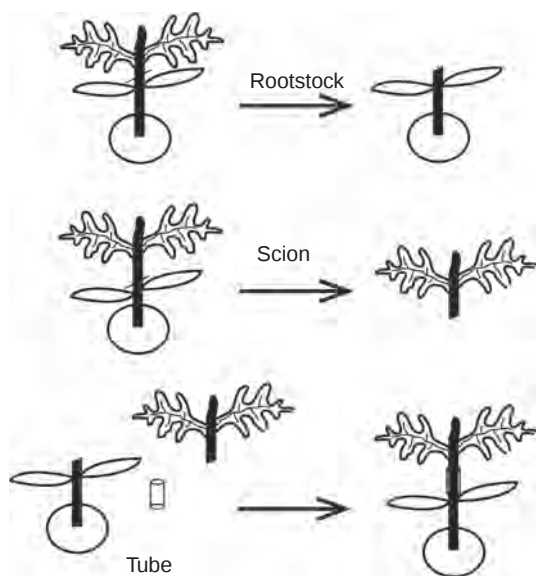


Fig. 14.3. Tube grafting of tomato (*Solanum lycopersicum*).

should both be at the two true leaf stage and their diameters must match (1.5 mm). The rootstock is cut using a razor blade at a 45° angle just above or below the cotyledons. Cutting below the cotyledons is preferred to prevent possible growth of axillary buds. A grafting tube (commercially available, made of silicone) is placed so that half of the tube is on the rootstock hypocotyl (stub remaining after cutting). The scion is cut at the same angle about 5–10 mm below the cotyledons, making sure the scion hypocotyl diameter matches the cut rootstock. The scion is placed in the grafting tube already on the rootstock, making sure the scion and rootstock cut surfaces align and fit tightly. The grafted seedling must be misted regularly to prevent desiccation during healing which occurs over a 7 day period.

Cucurbit grafting

Cucurbit (watermelon (*C. lanatus*), cucumber (*C. sativus*), muskmelon (*C. melo*)) rootstocks are selected for resistance or tolerance to *Fusarium* wilt and melon necrotic spot virus as well as tolerance to cool temperatures. Cucurbits are usually grafted to interspecific squash hybrids (*Cucurbita maxima* × *Cucurbita moschata*), bottle gourd (*Lagenaria siceraria*), figleaf gourd (*Cucurbita ficifolia*), or other melons (*C. melo*). Small trials for the best

scion–rootstock combination should be carried out since fruit quality and scion growth can be greatly affected by rootstock.

Watermelons (*C. lanatus*) are often grafted to interspecific squash hybrids to improve tolerance to chilling. Such plants may be too vigorous and may have delayed flowering and reduced sugar content of fruit. Thus they are often grafted to bottle gourd instead, which is less vigorous but still has chilling resistance. Muskmelons are usually grafted to interspecific squash hybrids or other muskmelons. When grafted to other muskmelons, identification of rogue rootstock fruit may be difficult. Cucumbers are normally grafted to interspecific squash hybrids or figleaf gourd. Some rootstocks improve cucumber fruit quality by reducing silicon deposition on the fruit epidermis (the bloom often seen on cucumber, which is not desirable).

Cucurbits are grafted using one of three methods: (i) hole insertion grafting; (ii) one cotyledon grafting; and (iii) approach grafting.

In hole insertion grafting, the scion and rootstock diameters do not have to match but the scion diameter must be the same or smaller than the rootstock. Scions are usually grafted onto rootstock cuttings rather than onto intact plants. For success, the graft union must heal and the rootstock cutting must form roots. The scion should be at the beginning of the first true leaf stage, around 2–3 mm. Rootstock seedlings should also be at the first true leaf stage (2 cm) with long hypocotyls (7–9 cm). Rootstock cuttings should be harvested about 30 min before grafting to allow them to lose some turgidity. This prevents cracking of the hypocotyl when the hole for insertion of the scion is made. The first true leaf, the apical meristem and any axillary meristems must be removed from the rootstock cutting. A hole is made in the rootstock cutting by inserting a sharpened chopstick or similar device into the axil of one cotyledon and forcing it through the hypocotyl, just below the opposite cotyledon. Cut each side of the scion hypocotyl making a cut edge about 7–10 mm long, but do not create a V at the end of the scion, but rather leave it somewhat wider than a V would be. Remove the tool used to form the insertion hole and insert the scion, cutting into the rootstock cutting making sure the cut surfaces of the scion make good contact with the rootstock tissue. Put the grafted cutting into a propagating tray filled with moistened media. Mist the cuttings to prevent dehydration.

Move the tray to a healing chamber for about 1 week to allow graft healing and rootstock rooting.

A second approach to cucurbit grafting is called the one cotyledon method, originally developed for robotic propagation operations. In this type of propagation, the rootstock may be an intact plant or a cutting. Both the scion and the rootstock should be at the first true leaf stage. Long rootstock hypocotyls (7–9 mm) are preferred if rootstock cuttings rather than intact plants are used. Remove one cotyledon from the rootstock at a 45° angle while at the same time removing the apical and both cotyledonary axillary buds. Prepare a scion with matching hypocotyl width by cutting at a 45° angle about 10–15 mm below the cotyledons. Make the cut perpendicular to the cotyledons so that when the scion and rootstock are grafted, the scion and rootstock cuts are aligned and one of the scion cotyledons is aligned with the rootstock cotyledon. Hold the graft together with an appropriately sized grafting clip. If utilizing rootstock cuttings, place the newly grafted cutting into new substrate and mist to prevent dehydration. Grafted plants should be misted as well to prevent desiccation. Move the cuttings or plants to the healing chamber for 7–10 days. The grafting clip can be removed once the graft has healed.

Approach grafting can also be used with cucurbits and while slower than other methods, it normally leads to a high success rate even with unfavorable healing conditions. Both the rootstock and the scion should be at the first true leaf stage. Using a sharp razor blade, make a downward cut at a 60° angle from horizontal about half way through the rootstock hypocotyl. Make a similar cut, only upwards, in the scion hypocotyl. Insert the downwards-facing tongue of the scion into the upwards-facing tongue of the rootstock. Hold the graft in place with a grafting clip. Immediately plant both plants in the same container and allow the graft to heal for 1 week. Once the graft has healed, remove the shoot of the rootstock and the roots of the scion.

Eggplant grafting

Grafting eggplants has not been as widely developed as tomato and cucurbit grafting, especially in North America. The rootstocks used for eggplant grafting include eggplant (*S. melongena*), torvum (*Solanum torvum*), and scarlet eggplant (*Solanum aethiopicum*).

Healing grafted seedlings

Graft healing is a critical step in the propagation chain of events and conditions should be maintained as close to those recommended as possible. Specialized chambers or greenhouses are used for healing. The relative humidity (RH) surrounding grafted cuttings or plants should be 95% or higher. As wounds heal, the RH can be allowed to decrease. Grafts should be kept within the temperature range of 28–29°C to stimulate callus formation at the graft union. Grafts should be kept in the dark for 24–48 h after grafting then provided with light either naturally or artificially at a level of 100 $\mu\text{mol}/\text{m}^2/\text{s}$ PAR. This light level is extremely low, close to the light compensation point which is just enough to maintain the plant during healing. Tomatoes require 4–6 days and cucurbits require 7 days to heal under these conditions.

Mukibat Grafting

One of the most intriguing types of grafting is called Mukibat grafting of cassava (*Manihot esculenta*) which was developed by a peasant subsistence farmer from Indonesia named Mukibat in 1958 (Mudge *et al.*, 2009). While most grafting is performed to establish a rootstock effect on a scion, Mukibat grafting is used to establish an invigorating scion effect on the rootstock. In cassava, the roots are harvested for food. Mukibat grafted a relative of cassava, the Ceara rubber tree (*Manihot glaziovii*) onto cassava leading to a 30–100% increase in the yield of cassava compared with non-grafted controls (De Bruijn and Dharmaputra, 1974).

Flower Bud Grafting

Flower bud grafting is a grafting technique adopted by the Taiwan pear industry (Kuniyama, 1996; Gemma, 2002). Asian pears (*P. pyrifolia*) are a favorite fruit in Taiwan, however, some desirable cultivars cannot be grown due to insufficient winter chilling to break dormancy. To overcome this production obstacle, budwood from trees in Japan that have received almost enough chilling to break dormancy is harvested, shipped to Taiwan then held at 2–4°C to complete chilling. The chilled buds are grafted onto ‘Heng Shan’ pear trees to produce two to four fruit per cluster that demand very high prices. The process is labor intensive and must be performed every spring.

***In Vitro* Grafting**

In vitro grafting is the technique where a 1.5 mm shoot tip from a mature plant is grafted onto a seedling rootstock (Murashige *et al.*, 1972). The technique was developed for citrus but is used for many species. Shoot tip grafting is used to clean cultivars of viruses. Plant meristems are free of viruses and other pathogens. When a desired cultivar is infected, clean plants can be produced by grafting meristems from infected plants onto a desired rootstock. The meristem that is grafted is free of the offending pathogen and, since it is from a mature plant, retains its mature status and does not have to go through a period of juvenility before it begins producing fruit. This type of grafting avoids the juvenility present in virus-free seedlings produced from clonally propagated nucellar seeds and cleans stock of pathogens that are resistant to thermotherapy cleaning (Roistacher, 2004).

Natural Grafting

Natural grafting, particularly root grafting, is common. While not used in commercial horticulture, natural grafting has some important ramifications on several economically important horticultural crops.

Two important ornamental diseases, dutch elm disease, caused by the fungus *Ophiostoma ulmi*, and oak wilt disease, caused by *Ceratocystis fagacearum*, are both transmitted via natural root grafts between individuals within each species (Epstein, 1978). Variegated chlorosis (*Xylella fastidiosa*) is a bacterial disease of citrus that can be transmitted between rootstocks of adjacent citrus trees through natural grafts (He *et al.*, 2000). Avocado (*Persea americana*) sunblotch disease (Mudge, *et al.*, 2009) and xyloporosis of citrus (Epstein, 1978) are diseases caused by a viroid and a virus, respectively, and are transmitted by natural root grafts.

While some consider the 'graft' between mistletoes (parasitic plants of the order *Santalales*) and their host plants natural grafts, they differ from true grafts in one very important aspect. In 'true' grafts, there are apoplastic connections between scion and stock as well as symplastic connections via plasmodesmata (Tidemann, 1989). In the 'graft' between mistletoe and host, no such plasmodesmatal connection has been observed (Coetzee and Fineran, 1989).

Graft Chimeras and Hybrids

One of the interesting aspects of grafting is the possibility of direct genetic transformation of one of the grafted components by another grafted component, known as a graft hybrid. Graft chimeras are often confused as hybrids, but they are not, as seeds produced by such chimeras do not segregate but rather reflect either one or the other of the chimera parents. Graft chimeras occur when the tissue of two distinct genetic components intermingle to present the impression that a new, often abnormal, curiosity has been formed by their union. Examples are often cited such as a lemon grafted to an orange which then produces fruit that are half sweet, half sour or a white rose grafted to a red rose which then produces both red and white flowers.

Graft hybrids would require actual genetic transformation of one tissue, usually scion, by another, usually rootstock. The transformed tissue would then have to produce seed that segregates into the characteristics of both 'parents'. Claims of such grafting-induced genetic transformations have not generally been reproducible. Even though reports have appeared in peer-reviewed scientific journals providing evidence that it does occur, no definitive proof has been offered and the subject remains highly controversial.