Grafting Manual:
How to Produce Grafted Vegetable Plants

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Authors:
Chieri Kubota
The Ohio State University

Carol Miles
Washington State University

Synopsis:
Healing is the most critical process of vegetable grafting propagation. Close attention to principles of successful healing is needed for good results.

Editors:
Chieri Kubota (The Ohio State University)
Carol Miles (Washington State University)
Xin Zhao (University of Florida)

This material is based upon work that is supported by the National Institute of Food and Agriculture, under award number 2016-51181-25404. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

USDA United States Department of Agriculture National Institute of Food and Agriculture

Healing and acclimatization methods and design principles

Healing is the most critical process of vegetable grafting propagation. Just like intensive medical care facilities are used after surgery in hospitals, the best results in healing can be obtained in a highly controlled environment as freshly grafted plants are vulnerable to temperature and moisture stress. However, simple tunnel-based systems can also provide good results as long as growers understand the principles for successful healing and pay close attention to the microclimate inside the tunnel.

Critical environmental factors

The three key factors for successful healing are 1) temperature, 2) relative humidity (RH) and 3) light. While there is a narrow optimum window for temperature and RH to achieve the best results, a wider range of light seems to be acceptable as long as the light level is not too high (to induce wilting) or too low (to induce stretching). However, the greater control you have to maintain targets for these three factors, the higher and more consistent the grafting success rate becomes. Grafting methods and scion/rootstock combinations also interact with these environmental factors to influence results. For example, an approach grafting method seems to be more successful than other methods under sub- or super optimum conditions and this is why small growers who have limited resources prefer this rather time-consuming grafting method. Also, cucurbit plants are more sensitive to healing conditions than tomato plants. However, some rootstocks of tomato are more sensitive to substrate moisture content during the healing process, and healing success is best when substrates are not too wet.

Optimum healing temperature should be selected so that the graft union develops a callus bridge (the layer of tissues acting as the interface between the scion and the rootstock) as quickly as possible. Once the callus bridge develops, plants can take up water more easily without the risk of wilting. This process of developing a callus bridge appears to take 3 days for tomato and 4-5 days for watermelon under optimum temperatures.

A study at Osaka Prefecture University, Japan showed that stomata of grafted cucumber plants began opening after 4 days in healing (Fig. 1), about the time the callus bridge is believed to be in place. Based on trials at the University of Arizona, plant temperature of 82-84°F (28-29°C) seems to consistently achieve a fast healing for both tomato and cucurbit species. When temperature is lower, healing takes more days.

While air temperature is controlled in healing, plant temperature is more important than air temperature as plant temperature could be higher than air temperature by a few degrees under solar radiation than under electric lighting especially when plant transpiration is limited (per leaf energy balance). Air temperature inside the healing chamber is often higher than air temperature outside the chamber by a few degrees when the chamber is within a greenhouse or high tunnel, or when structures holding the plants are covered with plastic (such as described below for indoor facilities). This may explain the conflicting temperature recommendations for healing from different scientists and growers, and it would be worthwhile noting the type of healing conditions each used to better understand the plant temperature in each system. A hand-held sensor with a thin thermocouple (0.2-0.5 mm in diameter) can be used for a quick check of plant temperature. An infrared gun is not recommended, as they tend to be inaccurate unless the user fully understands the

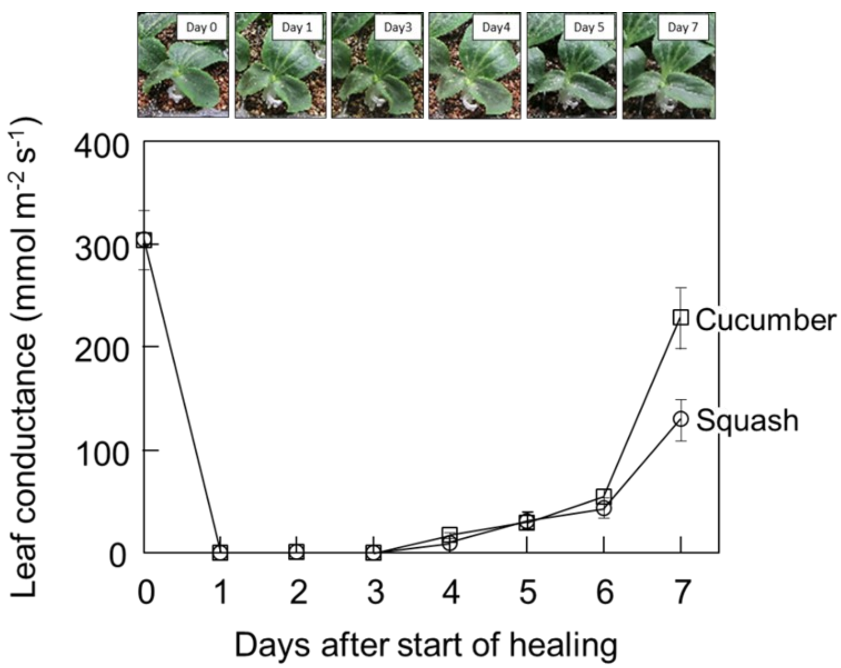

Figure 1. Stomatal conductance of cucumber cotyledons during healing after grafting onto squash rootstock or cucumber (self-grafted). Healing was done at 82°F (28°C) and 95% RH with 12-h per day lighting at 100 μ mol·m^{-2}·s^{-1} photosynthetic photon flux (T. Shibuya, unpublished data; Shibuya et al., 2015). Stomatal conductance was first measured on day 0 (the day before grafting) as a baseline. The first 3 days after grafting, stomatal conductance was essentially zero, suggesting that stomata are closed during this time. On day 4 after grafting, plants began to transpire, suggesting the functional development of a callus bridge between the scion and rootstock tissues.

physics of infrared measurements and correctly calibrates the sensor.

Relative humidity is most critical during the time when the callus bridge is not yet established. In general, tomato is less sensitive to suboptimal levels of RH than eggplant, and watermelon tends to be very sensitive and thus requires a more controlled environment (Johnson and Miles, 2011). Even though stomata are fully closed, scion cuttings can lose water through cuticular layers and eventually wilt. Maintaining very high RH (nearly saturation) is recommended during this period to minimize cuticular transpiration, and then gradually reducing RH to ambient levels once the critical time is past (~4 days for tomato and ~5 days for cucurbit plants after grafting at optimum plant temperature). Prolonged exposure to 100% RH may lead to problems such as fungal disease, adventitious rooting, and excessive stretching of the grafted plants.

While light is necessary for healthy plant growth, during the time the callus bridge is forming, only minimum light is needed. Some growers place newly grafted plants in darkness or in heavy shade conditions, or allow light only for a short period of time each day for the first 2-3 days following grafting. However prolonged darkness may cause excessive stretching of the plants. Very few growers have access to a light sensor to measure the actual amount of light and therefore it is difficult to find the actual range of light intensity they are using. However, it is unlikely to have a light intensity greater than 100 μ mol·m^{-2}·s^{-1} photosynthetic photon flux (the unit for photosynthetically active radiation) when electric lighting is used. In the greenhouse (or high tunnel), shading over the healing tunnel is often used to avoid wilting and to control temperature. Therefore, the actual range of light intensity during the healing conducted in a greenhouse is not well documented. The human eye is not reliable for judging light intensity inside a shaded structure especially when the structure is placed

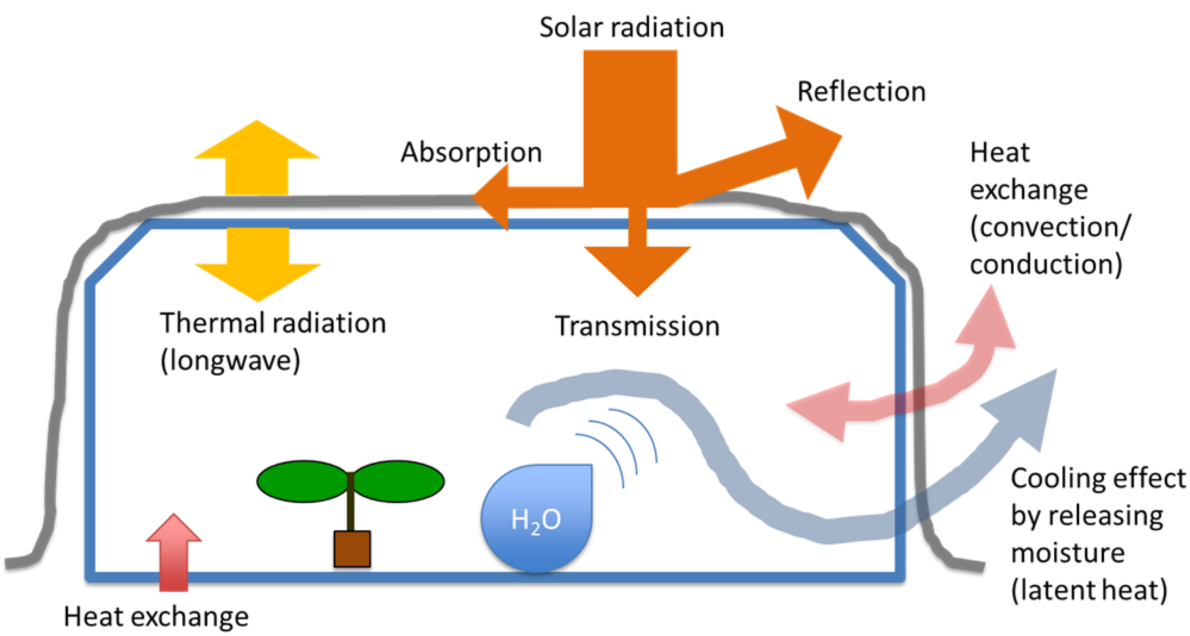

Figure 2. Thermal balance of a low tunnel style healing chamber exposed to solar radiation inside the greenhouse or tunnel. The key components to consider are 1) solar radiation and its transmission and absorption at the surface of the covering material, 2) thermal radiation reemitted from the surface of the covering material, 3) thermal exchange via convection and conduction at the surface of the covering material, 4) ventilation and associated evaporative cooling by releasing latent heat, and 5) heat exchange via the floor of the chamber.

within a bright greenhouse environment, and so observational information has limited value.

Understanding the energy balance of a healing chamber placed in a greenhouse or high tunnel (Fig. 2). would help growers to design a better healing system and to manage it to achieve the optimum healing conditions. Solar radiation affects the air temperature of the chamber and the plant temperature. Venting humid air from the chamber can effectively reduce the air temperature within the chamber (i.e., evaporative cooling) but ventilation must be managed to maintain a high level of RH inside the chamber. The internal conditions of a healing chamber that is exposed to solar radiation will fluctuate greatly due to the changing weather conditions (solar radiation, air circulation, air temperature and RH inside and outside the greenhouse or high tunnel). This is why standard operating procedures for healing chambers are site-specific or grower-specific. However, the key principles described here will provide growers and extension personnel the basis for developing their own successful protocols.

Design principles per healing system type

Low tunnel or tent inside a greenhouse or high tunnel: The key components for building a healing structure inside a greenhouse or high tunnel are:

1) Hoops or a frame to hold plastic and shade cloth
2) Clear or semi-transparent plastic cover to increase RH
3) Shade cloth to reduce light intensity
4) Fogging or misting system, or standing water for humidification
5) Heating system and thermostat control to maintain temperature within the desirable range when the ambient temperature is not optimal

Fig. 3 and Fig. 4 show some examples of low tunnel and tent healing structures placed inside a greenhouse or high tunnel. When this type of healing system is used, growers need to closely monitor and maintain the microclimate within the healing structure and not just within the greenhouse or high tunnel.

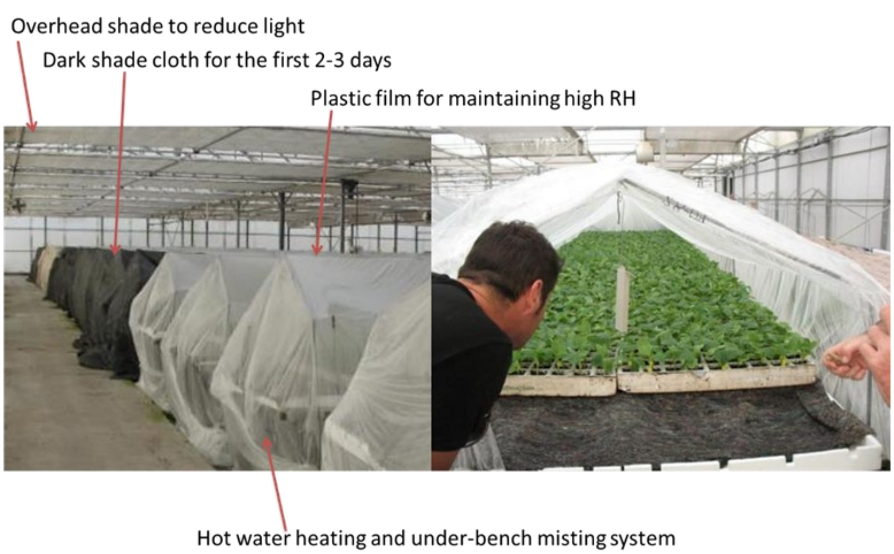

Figure 3. A bench-top healing system inside a greenhouse or high tunnel, commonly used by commercial nurseries. Overhead shade inside the greenhouse is used for better temperature control of the greenhouse and black shade cloth over the healing system is used for reducing light intensity. An under-bench misting system provides humidification without directly wetting the plants and hot-water bench heating keeps the plant temperature at an optimum range. Wetting the floor of the healing chamber is another technique commonly used to provide high RH without directly wetting the plants. (*Photos by Chieri Kubota*)

Indoor systems using electric lighting: Key components for designing an indoor healing chamber that can be placed inside a warehouse are:

1) Thermally well-insulated walk-in structure
2) Multi-tiered shelving units (movable or permanent)
3) Lamps
4) Humidification system
5) Cooling and heating system

Although consistency of healing conditions is achieved with this system, the capital investment and operational electricity costs are often discouraging. For example, a walk-in healing system with a capacity to hold 176 trays in multi-tiered shelving units was estimated as $13,193 to build and $0.0052 per plant per week to operate (at a density of 200 plants per tray, 35,200 plants total) when electricity was $0.09/kWh (Lewis et al., 2014). Lamps are a significant portion of the capital costs as well as the main user of electricity in this system. Therefore, selecting an efficient lamp system such as LEDs can reduce the operational costs but likely will increase the capital costs as the market price of LEDs is currently higher than conventional fluorescent lamps.

A typical design challenge is operation of electrical appliances and light in a wet environment. One solution commonly used in commercial operations is to cover the shelving units with a clear plastic film for humidification instead of fogging and misting inside the healing facility (Fig's. 5 and 6). Use of dry fogging is also preferred to standard fogging or misting to minimize surface wetness. Further optimization of the healing environment may improve the quality of plants coming out of the healing system. It may be worth considering a sophisticated computer-based algorithm for controlling the environment. Such an algorithm was developed to acclimatize vulnerable tissue cultured plants, where temperature and RH were controlled following a cyclically oscillating sine-curve function and increasing amplitudes over time from the starting set point and ending set point (Kozai et al., 1987).

Small-scale chambers: The key principles to follow to manage a healing system for

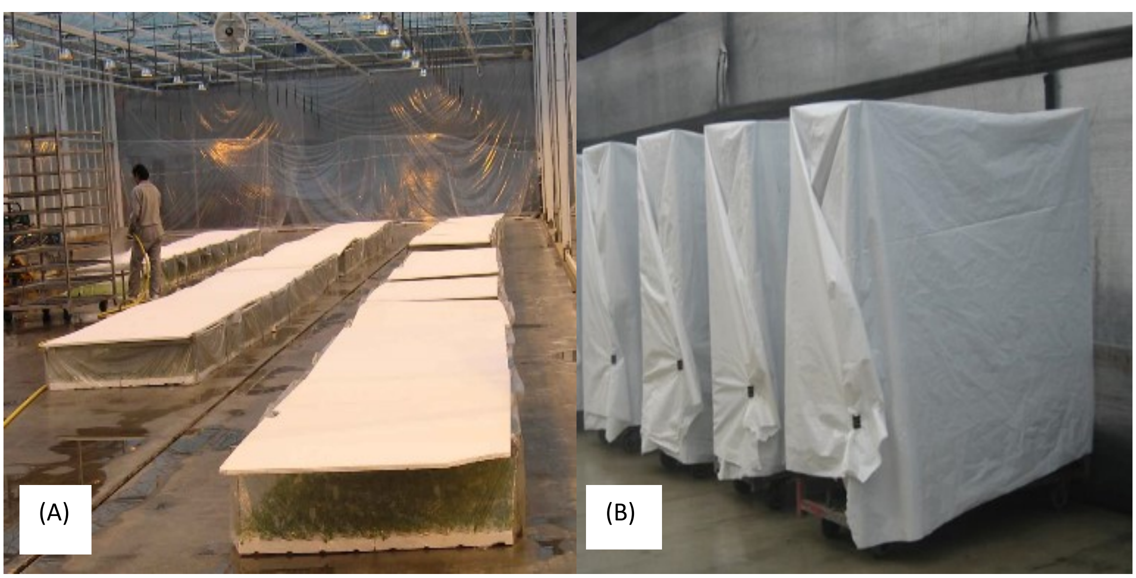

Figure 4. Various healing systems designed to place inside a greenhouse. (A) Plastic "tent" system to maintain the high humidity. Styrofoam panels are placed over the plastic tent for the first few days to reduce the solar radiation; (B) Nursery carts covered with white plastic sheets to reduce the light and increase the humidity. *(Photos by Carol Miles)*

very small applications are the same as those mentioned above. When the primary goal is to produce a small number of plants at low cost, a slightly lower success rate is not as critical. Examples of healing systems for small applications are shown in Fig. 7.

Hygiene practices

Regardless of the type of healing system used, hygiene practices including preventive measures to manage the risk of fungal diseases during healing are critical for successful healing. In some commercial grafting nurseries, steam is applied to sterilize the healing facility

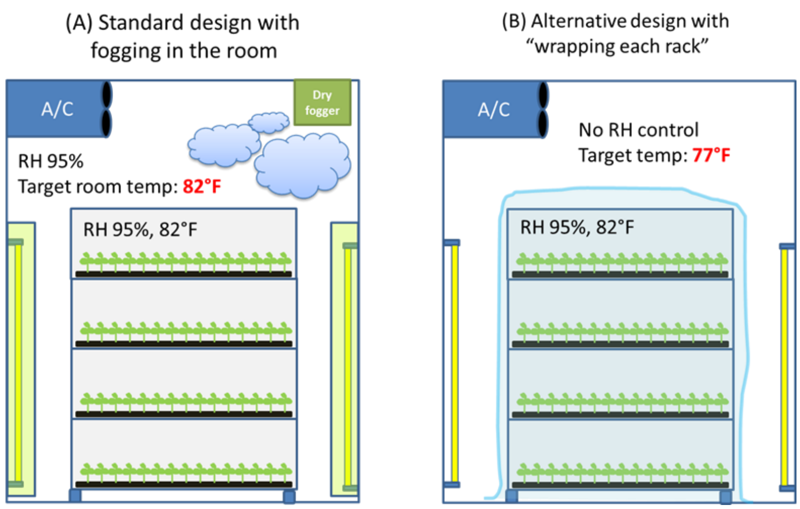

Figure 5. Two methods used to create high RH environment inside an indoor walk-in healing chamber. (A) Use of dry-fogging with lamps protected from moisture; (B) Use of plastic sheeting to wrap around the shelving units to increase the humidity without using fogging. The air temperature inside the chamber is lower for System B as the additional containment (plastic wrapped shelving units) would cause the temperature surrounding the plants to increase.

Figure 6. A healing facility developed in a commercial grafting nursery, with lamps protected with water-proof sleeves. *(Photo by Chieri Kubota)*

before and after use, and fungicides may be applied prior to healing if disease incidence is high. A 10% bleach solution is also used to sterilize benches, plastic, trays, grafting clips, and other supplies used for grafting. Minimizing the time that high RH is used in healing can minimize the likelihood of disease occurring in the healing facility.

Acclimatization after healing
Plants coming out of a high RH and low light environment need a few more days to fully acclimatize to the ambient environment inside the greenhouse or high tunnel. Grafted tomato plants tend to acclimatize more quickly than grafted eggplant and pepper (2 to 4 days, respectively), and grafted watermelon and other cucurbit crops tend to take the longest to acclimatize (up to 5-6 days) (C. Miles unpublished data). Following acclimatization, plants need to be hardened (2-3 days of reduced watering and exposure to outside temperature) before shipping or transplanting to the open field.

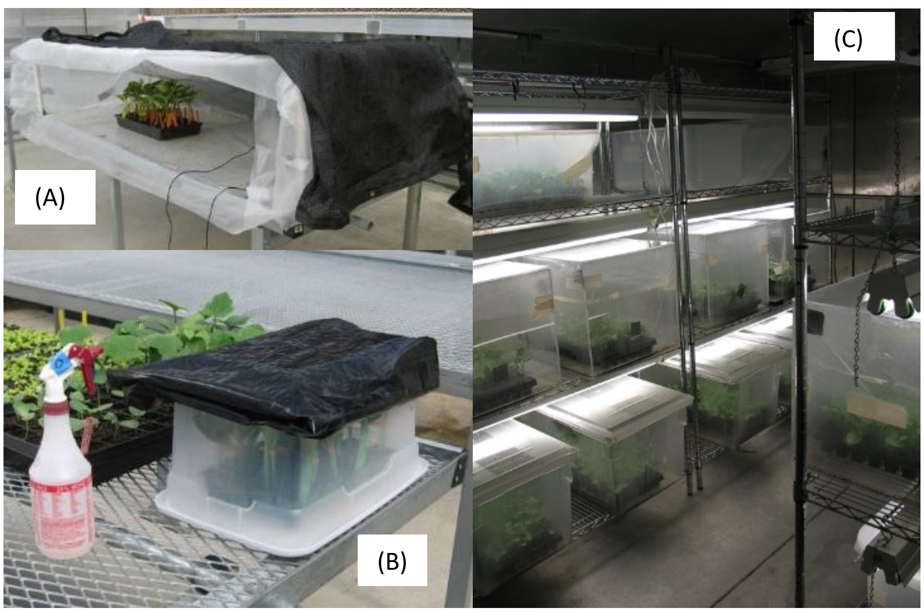

Figure 7. Healing chambers for small scale production. (A) A bench-top system developed at Washington State University (photo by Carol Miles); (B) A small upside down plastic container with a black plastic bag for home gardeners (photo by Carol Miles); (C) Small containers under fluorescent lamps used at the University of Arizona. If necessary, a thermostat-controlled heating mat can be placed under each container to maintain temperature inside the container. *(Photos by Chieri Kubota)*

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