NC STATE UNIVERSITY

Cary Rivard
Dr. Frank Louws
NCSU Dept of Plant Pathology

Grafting: A simple strategy for disease management in heirloom tomato production

Grafting vegetables for soil-borne disease resistance is an inherently simple process. Particular lines or cultivars are chosen for their genetic ability to resist, or tolerate soil-borne disease pressure. This line will be used as the rootstock. Next, a scion is chosen. The scion represents the above-ground portion of the plant, and it should be chosen based upon fruit quality. For heirloom growers, popular choices include "German Johnson", "Cherokee Purple", "Kellogg's Breakfast", etc. Shortly after germination, the rootstock and the scion are grafted together as seedlings. This takes place by severing each of the seedlings just above the cotyledons, and reattaching them so that the above-ground portion of the scion is secured to the root system of the rootstock. Once the grafted transplants are allowed to heal, they can be planted in the field, and managed similar to standard production systems.

Instituting New Technology With an Old Technique

Although the utilization of grafting in heirloom tomato production systems is relatively new, its foundation lies in an old principle. The first evidence for soil-borne disease reduction using grafts appeared in the early 20th century to diminish *Fusarium oxysporum* on watermelons. More recently, this technique has been used in an array of model disease management systems, where resistance genes have been tightly coupled to fruit quality genes. Possibly the most cited of these examples is the use of grafted tomatoes to reduce bacterial wilt (*Ralstonia solanacearum*). This disease complex has been particularly difficult to manage due to its wide host range and ability to persevere through long rotations. More importantly, breeders face numerous challenges in uncoupling the association between disease resistance and poor fruit quality. However, the worldwide implementation of grafting has significantly decreased the occurrence of bacterial wilt, while keeping fruit quality high. Furthermore, these challenges have resulted in a management practice that can be used wherever growers need to increase disease resistance, while keeping fruit quality at a maximum in order to compete in fresh-produce markets.

Figure 1 – Rubber tube-shaped clips are used in "tube-grafting"

The advent of "Japanese tube-grafting" has brought the practical application of using grafted tomatoes to the forefront due to its ease and high-throughput ability. Because many of the open-pollinated lines do not have good, inherent resistance, they cause serious problems in the field as disease epidemics may lead to total crop failures. Furthermore, those interested in this particular niche market are not willing to give up the increased fruit quality that goes along with heirloom cultivars. This unique situation has given rise to the idea that grafting may be able to increase disease resistance in heirloom production systems, without the use of chemical fumigants or other conventional methods. The purpose of this publication is to convey the potential of on-farm grafting to sustainable heirloom tomato growers as well as to explain the process of grafting in detail so that the readers may be able to perform this technique on their own farm.

Potential Benefits of Grafting

The benefits of grafting can be seen both directly through disease resistance as well as indirectly through increased water and nutrient uptake. Genetic resistance of rootstock can be very effective at decreasing the disease incidence of certain soil-borne pathogens. Disease resistance is a genetic trait, and although the rootstock provides the scion with a better conduit for below-ground activity, this practice does not alter the genetic make-up of the scion. This fact has two important implications. First, fruit quality will not be lost even though disease resistance has increased. Furthermore, the inherent resistance in the rootstock will not be transferred to seed produced by the scion. Therefore, seed harvested from the scion will not be any different from that collected from conventionally grown plants.

Disease Resistance

Research has shown that grafting can be effective against a variety of fungal, bacterial, viral, and nematode diseases. This technique has been used to eliminate *Verticillium* and *Fusarium* Wilt in melon and cucurbit production systems in Japan, Korea, and Greece (Oda, 1999: Ioannou, 2001). In New Zealand, grafted tomatoes were able to reduce levels of corky root rot (Bradley, 1968). In Morocco, grafting is used commercially to control root-knot nematodes (*Meloidogyne* spp.) and other soil-borne diseases in over 2000 ha of greenhouse tomato, melon and watermelon. In this region, grafting has replaced the use of methyl bromide as a way to manage soil-borne diseases (Abdelhaq, 2004). This method has also been successful for cucumbers against root-knot nematodes in Greece as well (Giannakou, 2003).

Grafting has been essential in Asian horticultural production for eliminating bacterial wilt incidence in solanaceous crops (Oda, 1999). It has also been used in tropical environments, like Brunei, where bacterial wilt incidence is so high that tomatoes cannot be planted unless the soil is sterilized or resistant rootstocks are implemented (Peregrine, 1982). In India, CRA 66 rootstocks were used to reduce bacterial wilt in tomatoes, and plant survivability rates at 1st harvest increased from 54.5% in the control to 100% (Tikoo, 1979). By the end of the season, none of the control plants had survived while 100% of the grafted plants continued to produce. Furthermore, the yield of the tomatoes with resistant rootstocks was 4 times that of the susceptible lines. This particular line was also identified for use against bacterial wilt in Germany and similar results were found (Grimault, 1994). Several Hawaiian lines (Hawaii 7996-7998) have been identified as suitable candidates for resistance to bacterial wilt (Oda, 1999). This technique could be a very valuable tool for eliminating bacterial wilt in tomato, pepper, and eggplant production systems.

Increased Crop Productivity

Grafting has been highly effective at overcoming abiotic stressors as well, which can indirectly lead to increased yield in a number of ways. Over 1/3 of all the irrigated land in the world is affected by salinity, and this technique could be instrumental in decreasing yield losses (Rivero, 2003). Grafting has also been utilized in order to reduce the effects of flooding in areas where a wet season may occur (Black, 2003). This technique has been shown to offer effective tolerance to soil temperature extremes. Because soil tends to heat and cool much more slowly than the aerial temperature, roots are exposed to extreme temperatures for a longer period of time than the aboveground structures (Rivero, 2003). In both of the cases, the growing season may be extended in either direction, resulting in better yield and economic stability through the year.

Although the use of grafted vegetables is associated with disease reduction and/or abiotic stressors, yield is often increased even without the presence of these identified stressors. Yields increased by as much as 106% compared to the control in watermelon production systems in Australia (Yetisir, 2003). Data which further supports this idea is sparse. However, in cases where lines have been bred specifically to be used as rootstocks, yield increases are evident (e.g. use of 'Maxifort' rootstock in greenhouse tomato production systems). This trend indicates the need for further research and development of this technique. By increasing yields even without the presence of specific disease pressure, grafting can be an economically viable method for improving production.

Carrying Out On-Farm Grafting

Know your farm

Probably the most important step to carryout successful disease resistance management in heirloom production systems is rootstock selection. In order to choose the right rootstock, the first step is to identify your potential pathogens. Farms which may have a long history of solanaceous crops (tomato, tobacco, potato, pepper) often have reoccurring problems with bacterial wilt. This disease has been particularly problematic in North Carolina, and has resulted in many farmers abandoning fields due to total crop failures. *Verticillium* and *Fusarium* wilt also continue to cause severe damage to tomato crops, especially in the mountain areas, where the climate favors the development of these pathogens. Root-knot nematodes are also a serious problem in North Carolina, and many times their development is difficult to diagnose before the problem has resulted in severe economic loss. By working with local state and county extension agents and educating yourself on basic disease diagnosis, you can easily identify potential pathogens that may hinder productivity.

Figure 2 – Commercial varieties of rootstock (HR=highly resistant, MR=moderate res, S= susceptible)

		Corky	Fusarium Wilt		Verticillium	Bacterial	
Rootstock	TMV	Root	Race 1	Race 2	Wilt	Wilt	Nematodes
Maxifort	HR	HR	MR	HR	HR	*****	HR
Beaufort	HR	HR	MR	HR	HR	*****	HR
Vulcan	HR	HR	MR	S	HR	S	MR
Magnet	HR	HR	MR	HR	HR	HR	MR
Seogun	MR	S	MR	S	S	S	MR
Shinmate	HR	S	MR	HR	HR	HR	MR
Joint	HR	HR	MR	S	HR	HR	MR

***** indicates no previous research. (adapted from Sakata, 2000)

Choosing the Rootstock

Rootstock selection should be based upon the potential for disease resistance. Many Asian and European companies are currently releasing varieties that have been bred for rootstock use. In Asia, Sakata Seed Company and Takii Seeds have an array of lines which have been released specifically for rootstock use. In Holland, De'Ruiter Seed Company has also released two cultivars ('Beaufort' and 'Maxifort'), which are meant for rootstocks. Although these cultivars have excellent disease resistance and many have increased yield even in the absence of disease pressure, their distribution in the United States is limited. Upon contact of these companies, it was found that the limited demand of these varieties has not warranted the added cost of the importation process into the United States. By contacting your local seed company, you may be able to make a difference solving this problem.

Although it would be ideal to find varieties that were specifically bred for rootstock production, the use of typical hybrids or "modern" varieties may also be valuable for heirloom producers. The key to choosing the right rootstock is to understand how to "break the tomato code". As breeders have developed lines with resistance, they have designated a

Scientific Name	Common Name	Traditional Code	2005 Internat'l Code
Tomato Mosaic Virus	Tomato Mosaic	Tm	ToMV
Tomato Spotted Wilt Virus	Spotted Wilt	TSWV	TSWV
Ralstonia solanacearum	Bacterial Wilt	R	Rs
Fusarium oxysporum f. sp. lycopersici	Fusarium Wilt (Races 0 & 1)	FF or F2	Fol: 0,1
Fusarium oxysporum f. sp. radicis-lycopersici	Fusarium Crown and Root Rot	Fr	For
Pyrenochaeta lycosersici	Corky Root Rot	K	Pl
Verticillium albo-atrum	Verticillium Wilt	V	Va
Verticillium dahliae	Verticillium Wilt	V	Vd
Meloidogyne spp.	Root-knot Nematodes	N	Mj, Mi, Ma

Figure 3 – Resistance Codes for all tomatoes have been recently changed.

code that can be displayed upon the label to identify these specific disease resistances. For example, a *Roma* VdFol:1 variety is one that has shown to be resistant to *Verticillium dahliae* and *Fusarium* (race 1), which are soil-borne, pathogenic fungi. Many of the modern hybrids have been bred to house resistance against a large number of pathogens. There are a number of resistance genes that have not yet been disseminated into modern breeding lines. For example, the best resistance for bacterial wilt lies in rootstock varieties available from Japan and India. By utilizing the resistance available in the "modern" tomato breeds, we may be able to induce resistance in some of our tastiest heirloom varieties.

Setting up the healing chamber

Although construction of a healing chamber is relatively simple and inexpensive, the proper location for the chamber on your farm may be somewhat difficult. While the grafts are healing in the chamber, it is best to ensure that they receive high humidity (80-95%), minimal direct sunlight, and a temperature range between 70 and 80°F. Furthermore, it is important that daily temperature variation remain low, as the formation of dew on the leaves can be detrimental to grafting success. Probably the best place for a healing chamber would be indoors in a heated storage area or garage, where fluorescent lights can be set up and turned on during the final days of healing. Healing chambers can also be maintained inside a greenhouse during the spring and fall so long as sufficient shading devices are set up to keep the grafts from excessive heat inside of the chamber.

A simple healing chamber consists of a frame covered by polyethylene sheeting, which can be used to keep the humidity levels high enough in order to ensure successful grafting. The floor of the chamber must be able to hold water, and during the first days after grafting, an opaque covering is used to keep all light out of the chamber.

At NC State, we have built an array of chambers to use for our grafting projects. Our most successful chamber is the simplest and most inexpensive. First, and most importantly, a tarp was stretched above the greenhouse bench to shade the area where the healing chamber would reside. This tarp was much larger than the chamber area in order to provide adequate shade throughout the day. Next, a layer of plastic sheeting was placed on the surface of the bench so that the raised edges of the bench were able to provide a shallow pool of water on the floor of the chamber. A frame was constructed using 1" PVC piping as illustrated below.

Figure 4 – Once grafts are place in the chamber, black plastic is used to keep out all available sunlight.

The frame was then covered in a layer of plastic so that the sides and end could be easily pulled up to check on the grafts during healing. Finally, black trashbags were cut longitudinally and stretched over the entirety of the chamber.

Planting the seeds

The grafting process itself and the subsequent healing time required mean that seeds should be sown 2 weeks before seeds are typically planted. This time will allow for up to one week in the chamber, and then 1 week in the greenhouse in order to acclimate to normal light conditions before transplants are put into the field.

In order to get good success during the grafting process, it is imperative that both the rootstock and the scion have stems with an equal diameter. Because different varieties require different germination periods, seeding times must be altered to get good grafting success rates. By staggering plantings, you can offset the effects of variable germination periods so that the seedlings are the same size on the day of grafting. In many cases, rootstock varieties take much longer (2-5 days) to germinate than typical heirloom breeds. Many times, it is a good idea to plant a few seeds “test seeds” of each of your varieties 2-3 weeks before you plant your seedling crop to find out how long the germination period is in your greenhouse or propagation facility.

Choosing the best time to graft

Tube grafting should be carried out when the seedlings have 2-4 true leaves, and the stems are typically 2-3 mm in diameter. In order for proper healing to take place, the vascular tissue in both the rootstock and scion are required to line up so that these tissues can easily grow back together, forming a strong union for water and nutrient uptake. This means that an essential component to grafting success is to have rootstock and scion that have similar stem diameters.

Figure 5 – Seedlings are grafted when 2-4 leaves are present and stem diameter is 2-3 mm.

Grafting should take place when there is little water stress upon the plants. Early in the morning or just after dark are excellent times to graft as the plants are typically transpiring slowly at these times. Additionally, it is important that the grafting process be carried out indoors or under some sort of shading device. If daytime grafting is essential due to timing and labor concerns, it is recommended that the plants be put in a shady area in the morning before transpiration increases to prevent unwanted water stress during the process.

Grafts are made by severing the rootstock and scion at a 45^o angle and reattaching them with a rubber or silicon clip. Although the specific degree of this angular cut is not essential, it is important that the cut be made at an angle, thereby providing more surface area for the vascular tissue to grow back together. Other methods have tried to increase this surface area even further by making V-shaped cuts, but their results are similar to those of the tube grafting technique.

Figure 6 – Stems are severed at a 45^o angle and reattached with a rubber clip so that good contact is kept between the rootstock and the scion.

Life in the chamber

Immediately after grafting takes place, the plants are required to from callus tissue and reform vascular bundles to provide the scion with nutrients and water. The purpose of the chamber is to keep the scion from getting water stressed while this process occurs. Decreasing water stress can be done by slowing the transpirational stream that is moving through the plant. The best ways to do this are to increase humidity, decrease light levels, and decrease temperature. However, by decreasing the temperature, you may hinder the development of newly-forming callus tissue. Therefore, the keys to good chamber operation are high humidity, and no direct sunlight. Humidity can be increased in a number of ways. Some of our chambers have implemented the use of cool-water vaporizers, which can be purchased at local drug stores. In small chambers, the most effective method of regulating humidity can be easily done by adding a pool of water to the benchtop. In this case, the natural ability of water to evaporate in warm temperatures will keep the chamber sufficiently humid.

Once your grafts have been made, it is important to put them into the healing chamber as soon as possible. It is best to have the humidity, light, and temperature levels constant before beginning the procedure so that the grafts can be placed immediately into the functioning chamber. There are a number of things that can happen inside the chamber that will greatly affect grafting success rates.

The first major constraint for proper graft healing is water stress. Because the scion have been physically separated from their root system, they may have a tendency to become wilted. Typically, a small amount of wilt during the first day of healing is acceptable, and it should decrease as humidity levels go up within the chamber. By carefully keeping this upper portion alive, you may give the graft a better chance of survival.

Any activity that pulls the scion stem away from the rootstock stem will decrease the contact surface between these tissues, ultimately decreasing graft success. Therefore, grafts should be carefully placed into the chamber, and should be subsequently inspected to be sure that the scion has not fallen away from the rootstock. Furthermore, water on the leaves can also pull the scions away from the rootstock. For this reason, **it is not recommended to mist grafts once they are in the chamber**. While the grafts are still weak, water should only be applied directly to the benchtop so that it can be taken up by the roots.

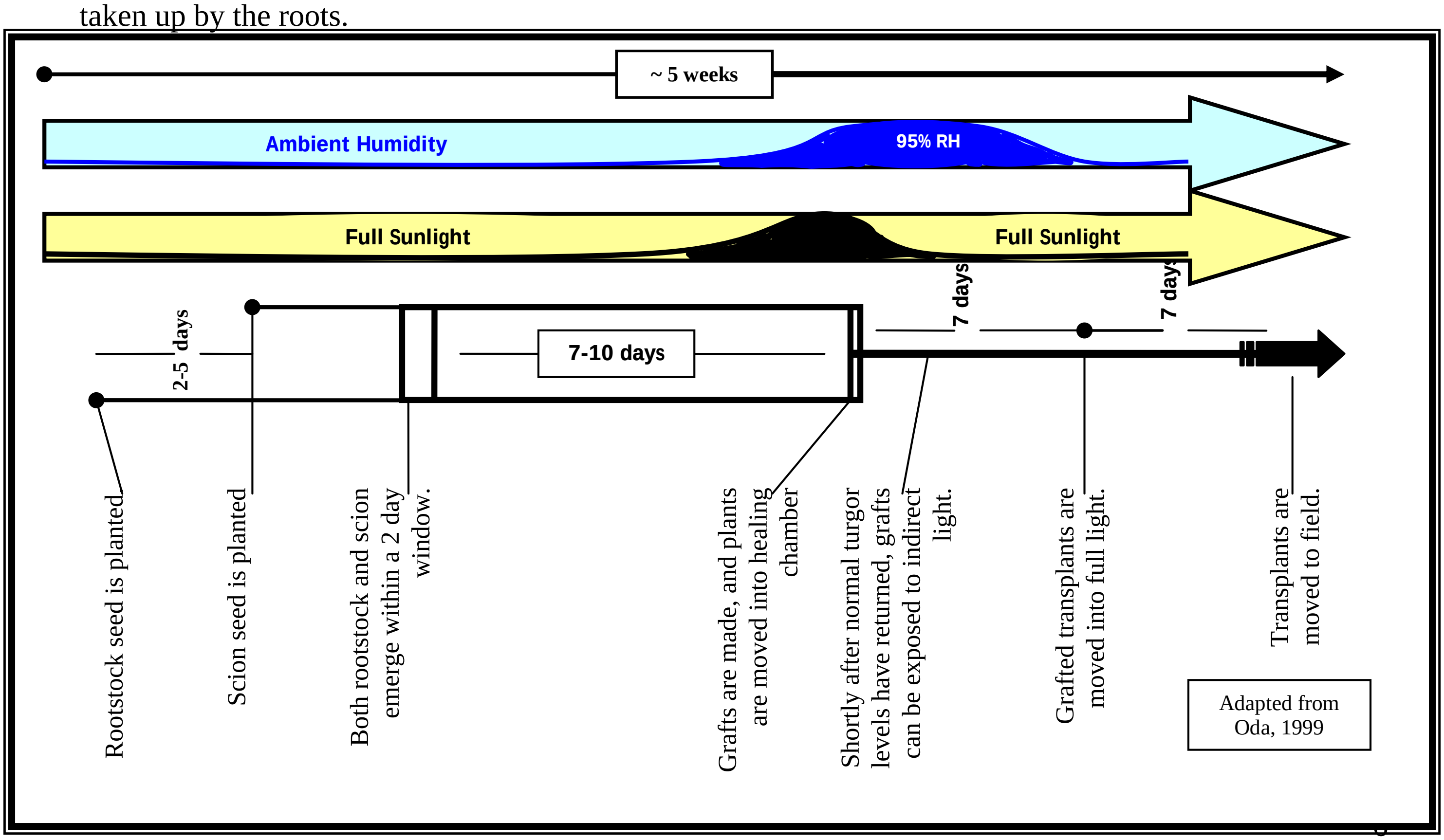

Figure 7 – A typical timeline for graft production.

Returning to life on the farm

Once the grafts have been made and placed into the chamber, they must be slowly acclimated to normal conditions. Grafts should be left in the chamber for the first 2-5 days with absolutely no light and high humidity. After these first few days, you will be able to notice grafts which were successful, and grafts that were not. The scion should be back up to normal turgor levels, and there should be no evident signs of wilt. Once you believe that the grafts have healed properly, you can begin to slowly introduce light, and decrease the humidity levels over the following week. Although it is impossible to make this process go too slow, it can easily be carried out too fast. When you first introduce light back into the chamber, it is best to simulate indirect light. If your chamber is inside, you can put a couple of fluorescent lights above the chamber. If your chamber is in a greenhouse, then you want to make sure that a shading apparatus is set up that will significantly reduce the amount of light without eliminating it altogether. Once the transplants have acclimated to this reduced light level, you can begin to decrease humidity by lifting up the sides of the chamber, allowing fresh air to move across the newly grafted plants. Shortly thereafter, the plastic sheeting can be completely removed, and the plants can be exposed to direct sunlight.

Figure 8 – Clip falls off after graft heals.

Because the grafts may still not be very strong, it is a good idea to be very careful while transporting and watering recently-healed transplants. Watering can be done from the bottom so that the scion is securely retained on the rootstock. Alternatively, careful methods can make top-watering possible as long as the scion is not exposed to high water pressure.

Figure 9 – Edema on tomato leaves.

Pitfalls of Grafting

Although grafting increases the disease resistance of plants in the field, the process itself may expose your transplants to situations that could favor disease in the greenhouse. For instance, by severing the stem of the plant, you provide an easy entry point for bacteria, fungi, and viruses to invade your tomatoes. Therefore, it is imperative to keep your working area, chamber, and all tools extremely clean during this process. Additionally, the standing pool of water in the chamber may lead to problems with pythium-related diseases. This can be avoided by preventing soil contamination and using light potting mixes that reduce the moisture level in the soil for transplant production. Grafts may form edema if they are allowed to stay in the chamber for too long. Edema is a physiological disorder that is a result of too much humidity. This is not a serious problem, but if the transplants are moved into an environment with lower RH, this problem should go away.

If the graft union was not able to keep good contact, it may become disfigured. This may cause problems in the field if the union is not strong enough to hold the weight of the vines. By being careful during planting and tying, this problem can be overcome in the field. Once the grafted tomatoes are in the field, they should be monitored to keep suckers from robbing the scion of water and nutrients. These suckers can easily be identified as branches that form below the graft union. Fruit that is borne from these branches will be the genetic result of the rootstock, and therefore unwanted. By trimming these suckers, you will provide the scion with more water and nutrients, ultimately leading to higher crop productivity.

Figure 10 - Poor contact leads to a disfigured union.

Summary

Grafting is a valuable management tactic for heirloom tomato growers. This practice originated as a way to ensure fruit quality, while keeping disease resistance high for melon, tomato, and cucurbit production systems with soil-borne disease pressure. This same principle lends itself well to organic heirloom production systems. Furthermore, increased yields have also been evident for many grafted vegetable crops. As Southeastern growers realize the potential and relative ease of this process, it will certainly become more popular as a disease management technique in the United States.

The author wishes to thank: Dr. Frank Louws, Dr. Mary Peet, Jim Driver, Rob Welker, Mike Carnes, and the NCSU Department of Plant Pathology. Edema photograph courtesy of Dr. Mary Peet (NCSU). All others prepared by the author.

Author contact information:

Cary Rivard
NCSU Dept of Plant Pathology
Raleigh, NC 27695
caryrivard@hotmail.com

Department of Plant Pathology

References

Abdelhaq, H. 2004. Integrated Production and Protection in Greenhouse Crops. *Extension Publication*. Institut Agronomique et Vétérinaire Hassan II

Black, L. 2003. Grafting Tomatoes for Production in the Hot-Wet Season. *InternationalCooperators' Guide*. Asian Vegetable Research & Development Center. pub #03-551, May

Bradley, J. 1968. Tomato Grafting to Control Root Diseases. *New Zealand Journal ofAgriculture*. 116:26-27.

Giannakou, I, D. Karpouzas. 2003. Evaluation of Chemical and Integrated Strategies as alternatives to Methyl Bromide for the Control of Root-Knot Nematodes inGreece. *Pest Management Science*. 59:883-892

Grimault, V, P. Prior. 1994. Grafting Tomato Cultivars Resistant of Susceptible to Bacterial Wilt – Analysis of Resistance Mechanisms. *Journal of Phytopathology-Phytopathologische Zeitschrift*. 141:330-334.

Ioannou, N. 2001. Integrating Soil Solarization With Grafting on Resistant Rootstocks for Management of Soil-Borne Pathogens of Eggplant. *Journal of HorticulturalScience & Biotechnology*. 76:396-401.

Lee, M. 1994. Cultivation of Grafted Vegetables. I. Current Status, Grafting Methods, and Benefits. *HortScience*. 29:235-239.

Oda, M. 1995. New Grafting Method for Fruit-Bearing Vegetables in Japan. *JapanAgricultural Research Quarterly* 29: 187-194.

Oda, M. 1999. Grafting of Vegetables to Improve Greenhouse Production. *ExtensionBulletin*. College of Agriculture, Osaka Prefecture University. December

Peregrine, W. 1982. Grafting - A Simple Technique for Overcoming Bacterial Wilt in Tomato. *Tropical Pest Management* 28:71-76

Rivero, R. 2003. Role of Grafting in Horticultural Plants Under Stress Conditions. *Food, Agriculture & Environment*. 1:70-74.

Tikoo, S. 1979. Successful Graft Culture of Tomato In Bacterial Wilt Sick Soils. *Current Science*. 48:259-260.

Vallad, G, R. Goodman. 2004. Systemic Acquired Resistance and Induced Systemic Resistance in Conventional Agriculture. *Crop Science*. 44:1920-1934.

Yetisir, H, N. Sari. 2003. Effect of Different Rootstock on Plant Growth, Yield and Quality of Watermelon. *Australian Journal of Experimental Agriculture.* 43:1269-1274.