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ADVANCES IN VEGETABLE GRAFTING AND NEW NURSERY PATTERNS FOR GRAFTED PLANT PRODUCTION

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1
Introduction

For the past, methyl bromide (MB) was the fumigant of choice for many replant soil applications. The reasons primarily focus on the broad spectrum of activity of the fumigant, its high vapour pressure facilitating distribution through the soil profile, cost-effectiveness, and comparatively short plant-back intervals. Historically, MB was primarily used to control lethal soilborne pathogens such as *Verticillium dahliae* (Wilhelm and Paulus AD, 1980), but it also can provide excellent control of nematodes and a broad spectrum of weeds. With the Montreal Protocol, that was established to regulate the use of gases with high ozone depletion potential (ODP) in order to protect the ozone layer from human pollution (Ristanio and Thomas, 1997; UNEP, 2006), from 1st of January 2008 MB was definitively banned for use as a soil fumigant in Italy. Italy, first in Europe and second in the world for the use of MB in horticultural crop production, completely replaced MB uses both by adopting chemical and not chemical solutions. Moreover new strategies were adopted by combining several control methods including grafting commercial cultivars onto resistant rootstocks. Even though grafting was practiced in fruit trees for thousands of years, vegetable grafting was only recently widely adapted on a commercial scale (Ashita, 1927; Sakata *et al*., 2007). Old records on vegetable grafting can be found in Chinese as well as in Korean and Japanese writings. The commercial use of vegetable grafting is a relatively recent innovation. The wide use of plastic films for the production of vegetables in the late 1950s, and the increased interest on protected cultivation, which involves successive cropping, determined the use of grafted vegetables. Commercial vegetable grafting originated in Japan and Korea and was practiced for about 30 years. It was introduced to Western countries in the early 1990s and is currently being globally practiced by using local scion cultivars and introduced rootstocks. Grafting vegetables represents a feasible

alternative, technically and economically accepted by growers in several growing conditions: 54% and 81% of vegetables grown in Japan and in Korea are grafted (Rivero *et al.*, 2003). In Mediterranean countries such as Greece, Spain, Netherlands, France, Cyprus, Malta and Italy rapidly increased the adoption of grafted plants (Traka-Mavrona, 2000; Diánez *et al*., 2007; Minuto *et al*., 2007). In Italy more than 25 millions of grafted plants were produced in 2012 including melon, watermelon, tomato and eggplant.

The high demand for off-season vegetables and the intensive cropping systems with limited crop rotations has determined a buildup of detrimental factors (biotic or abiotic) that can substantially reduce yield and crop quality. Grafting vegetables onto resistant rootstocks offers numerous advantages such as the tolerance or resistance to environments that are too cold, wet or dry, hypoxious, salty, and with heavy metal contaminations, excessive and insufficient nutrient availability, and soil pH stress (Abdelmageed and Gruda, 2009; Ahn *et al.*, 1999; Estañ *et al*., 2005; Venema *et al*., 2008; Rivero *et al.*, 2003a,b; Savvas *et al.*, 2010). Nevertheless resistance to soil pathogens, in particular *Verticillium* and *Fusarium* (Lee, 1994), is one of the most important increasing the use of grafted plants after methyl bromide ban.

Even though benefits of grafted seedlings use are widely recognized, the grafting technique has some limits: the rootstocks resistance may break down under high pathogen population pressure, the new races of pathogens may evolve, and, under some environmental conditions e.g. high temperature and salinity, the plants may collapse. Moreover minor pathogens can become major pathogens on the rootstocks, particularly when soil fumigants are not used in combination with this technology. In addition, grafting technique might cause physiological disorders. In Northern Italy sudden collapses were reported on protected and open field tomatoes (cv Cuore di Bue and cv Marmande-Raf) grafted on cvs He-Man and Energy (Garibaldi and Minuto, 2003). In Southern Italy sudden collapses were reported on cv Iride, Naomi, Cuore di Bue, and Marmande-Raf grafted on cvs He-Man, Energy and sometimes on Beaufort, without a direct relationship with the growing season or the phenological stage (Garibaldi and Minuto, 2003). Eggplants grafted onto *L. lycopersicum* x *L. hirsutum* and *L. lycopersicum* hybrids may show graft incompatibility during all phases of the growing season and particularly when plants are transplanted in cold seasons (autumn, winter

and early spring) and in not-heated greenhoses, resulting in plant dieback (Minuto *et al.*, 2007).

Improving crop productivity was always one of the major aims in agricultural development. In the last decades, however, crop quality was pursued as a more important feature than yield and it has become the first goal for modern agriculture. While major improvements in crop yield were obtained through selection for best harvest indexes and appropriate cultural techniques such as the grafting technique, increasing crop quality is strictly depending on the understanding of the growth mechanisms that lead yield organs are strictly related with the genotype. Sicily is the largest Mediterranean island located in southern Italy. It is a cultural and a commercial port, and one of the most important centre of origin and differentiation of vegetables. During the centuries, the farmers obtained many genotypes for each species, adapting them to the pedoclimatic requirements, and not caring of their to the genetic purity. For this reason, it was estimated a presence of 2650 taxa (Raimondo *et al.*, 1992) in Sicily on an extension of 26000 Km^2. This selection criteria allowed to obtain an inter-specific variability that brought other genotypes perfectly integrated with the cultural environment and with positive effects on the qualitative and organoleptic characteristics (Schiavi *et al.,* 1991). Breeding activity is always depended on the availability of genetic variability, and thanks to the selection criteria applied by the farmers the biodiversity was saved (Schippmann *et al.,* 2002). The local populations are genotypes of high intrinsic value with a particular capacity of adaptability to their environment. These characters allow easier cultivation in Mediterranean basin compared to the varieties selected in different environments. Consequently, during my doctorate in Environmental Agronomy, the research activities were focused on the recovery, discovery, and characterization of Sicilian ecotypes, and their use as scion, in order to improve quantity and quality yields. In addition, were carried out other researches concerning the manipulation of the traditional scheme of the grafting technology, in order to overcome problems related to the high grafted seedlings transport cost and the eggplant grafted seedlings nursery flexibility using seedlings propagated by unrooted grafted cutting.

1.1 Global overview on grafted plant production

Although the benefits of using grafted plants were known, the large-scale commercial growing of grafted vegetables can be traced from the late 1950s in Japan and Korea, where nowadays 721.3 million of grafted seedlings are distributed (Jung-Myung Lee *et al.*, 2010). In Korea, more than 100 seedlings growers, excluding individual farmers and farmers' associations, are producing plug grafted seedlings. Hoban Nursery, the largest nursery one in Korea, produced 15.6 million grafted seedlings in 2008. Nongwoo GreenTek produced 9.0 million grafted seedlings followed by Nosung, Gongju, and Yeoju (Jung –Myung Lee *et al.*, 2010). Pureun Nursery produced about 2.8 million seedlings, mostly for export, even though less than 10% of all grafted seedlings are estimated to be produced by commercial growers in Korea (Ko, 2008). Although vegetable grafting is practiced in many countries, accurate statistics are unavailable. However, 40–45 million grafted seedlings were distributed in North America in 2005 (Kubota *et al.*, 2008). Spain represents the leading European country in using grafted vegetable transplants (129.8 million in 2009) (Jung-Myung Lee *et al.*, 2010), followed by Italy (47.1 million) (Morra and Bilotto, 2009) and France (about 28 million). In the table 1 is reported a personal communication concerning the totally cultivated area of the most important vegetable crops, and the area transplanted with grafted seedlings.

Table 1 – Growing area in Italy (pers. commun.)

Crop	Totally cultivated	Area of grafted plants
Tomato	727878	109181
Eggplants	154890	23234
Pepper	241251	12063
Cucumber	66276	3313
Watermelon	9000	7200
Other melons	15000	7580
Total	1214295	162571

In addition, by observing the historical trend, a rapid increase in the use of grafted seedlings is expected throughout the world for some decades.

Several long term breeding program, carried out by many multinational seed companies, are supplying rootstock seeds which virtually have little or no negative effects on fruit

quality in some vegetables. The majority of the grafted seedlings are produced by commercial growers globally, and the price of grafted seedlings change with vegetable species and countries: from 0.4 $ to 1.2 $ per seedling in the USA and some Asian countries including Japan and Korea, from 0.6 € to 1.0 € per seedling in Spain and some European countries.

1.2 Purpose of vegetable grafting

1.2.1 Tolerance to soil-borne diseases

The roots of selected rootstock can exhibit excellent tolerance to soil-borne diseases, such as those caused by *Fusarium, Verticillium, Phytophthora, Pseudomonas, Didymella bryoniae, Monosporascus cannonballus*, and nematodes (Edelstein *et al*., 1999; Cohen *et al*., 2000, 2005, 2007; Ioannou, 2001; Trionfetti Nisini *et al*., 2002; Blestos *et al*., 2003; Morra and Bilotto, 2006; Crinò *et al*., 2007) even though the tolerance level varies with the rootstocks. The mechanism of disease resistance, however, was not intensively investigated. Resistant rootstocks can be also very useful in hydroponics system, to effectively counteract the rapid disease spread. The disease tolerance in grafted seedlings may be entirely due to the tolerance of rootstock roots to such diseases. However, in actual planting, adventitious rooting from the scion is common. Plants having the root systems of the scion and rootstock are expected to be easily infected by soil-borne diseases. However, seedlings having dual root systems occasionally exhibit a certain degree of disease resistance, thus partially supporting the previous report that substances associated with *Fusarium* tolerance are synthesized in the root and move to the scion through the xylem. It is generally accepted, however, that the disease susceptible characteristics of the scion are not transported to the rootstock.

1.2.2 Tolerances to abiotic stresses

Since the grafts were always used to induce resistance against low and high temperatures (Rivero *et al*., 2003; Venema *et al*., 2008), improve water use efficiency (Rouphael *et al*., 2008a), reduce uptake of persistent organic pollutants from agricultural soils (Otani and Seike, 2006, 2007), improve nutrient uptake (Colla *et al*., 2010a), increase synthesis of endogenous hormones (Dong *et al*., 2008), improve alkalinity tolerance (Colla *et al*., 2010b), raise salt and flooding tolerance (Romero *et al*., 1997; Colla *et al*., 2006a,b; Yetisir *et al*., 2006), and limit the negative effect of boron, copper, cadmium, and manganese toxicity (Edelstein *et al*., 2005, 2007; Rouphael *et al*., 2008b; Savvas *et al*., 2009).

Salinity is one of the most important abiotic stresses that influences plant growth and crop productivity in many vegetable production areas of the world (Colla *et al*., 2010).

Grafting can represent an interesting environmentally friendly alternative to avoid or reduce yield losses caused by salinity stress, in vegetables belonging to *Solanaceae* and *Cucurbitaceae* families. Grafting is an integrative reciprocal process and, therefore, both scion and rootstock can influence salt tolerance of grafted plants. Grafted plants grown under saline conditions often exhibited better growth and yield (Santa-Cruz *et al.*,2001), higher photosynthesis (Colla *et al*.,2006; Yang *et al.*, 2006) and leaf water content (Santa-Cruz *et al.*, 2002), greater root-to-shoot ratio (Huang *et al*., 2009), higher accumulation of compatible osmolytes (Munns and Tester, 2008), abscisic acid and polyamines in leaves (Liu *et al*., 2004), greater antioxidant capacity in leaves, and lower accumulation of Na+ and/or Cl− in shoots than ungrafted or self-grafted plants (Colla *et al.*, 2010).

Excessive levels of heavy metals in agricultural land constitute an increasingly serious threat not only for intact plant growth and yield, but also for environment and human health (An *et al*., 2004; Gratão *et al*., 2005; Clemens, 2006; Hong-Bo *et al*., 2010; Raskin *et al*., 1997). Some heavy metals are toxic to plants even at very low concentrations, while others may accumulate in plant tissues up to a certain level without visible symptoms or yield reduction (Clemens, 2001; Moustakas *et al*., 2001; Verkleij *et al*., 2009). Fruit vegetables, such as tomato, pepper, and eggplant, are characterized by rather low rates of heavy metal translocation to the fruit (Angelova *et al.*, 2009). Therefore, the impact of grafting on the uptake of heavy metals by fruit vegetables was so far hardly investigated. Arao *et al*. (2008) assessed that the grafting technique, using *Solanum torvum* as rootstock, is able to reduce the Cd concentration in eggplant fruits. In particular, the results obtained show an appreciable restriction of the Cd translocation from root to shoot. Genotypic differences in the ability of the root to prevent Cd translocation to the shoot were demonstrated also for soybean by means of grafting experiments (Sugiyama *et al*., 2007). Yamaguchi *et al*. (2010) attempted to elucidate the molecular mechanisms governing the reduced Cd uptake by *S. torvum* and found that dehydration-related transcription factors and aquaporin isoforms are potential constituents of Cd-induced biochemical impediments. Other results have shown that the rootstock significantly affects gene expression in the scion, thereby indicating that some signals transported from the root to the shoot may also influence the Cd uptake and translocation (Si *et al*., 2010). Edelstein and Ben-Hur (2007),

assessed the influence of the grafting on heavy metal concentrations in melon plant fruits ‘Arava’, using Cucurbita rootstock ‘TZ 148’. The results obtained show a lower level of B, Zn, Sr, Mn, Cu, Ti, Cr, Ni, and Cd metals in fruits from grafted plants than from ungrafted plants. The lower heavy metal and trace element concentrations in fruits were ascribed mainly to differences in characteristics of the root systems between the two plant types. Nevertheless, further research is needed to elucidate the mechanisms involved in impediment of heavy metal translocation from the root to the shoot in some rootstock/scion combinations.

1.2.3 Yield increases

Frequently the grafting is strictly associated with increases in fruit yield in many fruit vegetables (Sabatino *et al*,2013). The yield increase are closely correlated with the maintenance of good plant vigor until late in the growing season (Sabatino *et al*, 2013) in addition to disease resistance. Using Fusarium infected substrate, up to 54% increase of marketable yield in tomato was obtained with ‘Kagemusia’ and 51% with ‘Helper’ rootstocks (Chung and Lee, 2007). There was also a significant decrease in abnormal fruits in plant grafted onto most rootstocks as compared with the own-rooted ‘Seokwang’ tomato. Similar yield increase have been reported by other researchers on watermelon, cucumber (Lee and Oda, 2003), melon, pepper, and eggplant.

1.3 Rootstock

For each vegetable crop, different rootstocks were selected from the existing cultivars. Due to the increased interest on the cultivation in protected environment, the seed companies and various breeders, are eager to breed superior rootstocks for vegetables grown under specific conditions and environments. Therefore, the growers have to make decision on selection of rootstocks most suitable for their specific requirements. Nowadays the number of registered rootstock cultivars are rapidly increasing mostly due to the increased cultivation of grafted plants under various cultural as well as environmental conditions (Kato and Lou, 1989; Ko, 1999; Lee *et al.*, 2008). Frequently, rootstocks belonging to different species are preferred in order to exploit the genetic diversity. Hence, rootstocks of different species are used in cucurbits (Table 2) and solanaceous crops (Table 3).

Table 2 – Rootstocks for cucurbitaceous crop and some major characteristics.

Rootstock	Major characteristics[a]	Possible disadvantage
Watermelon		
Bottle gourd (*Lagenaria siceraria* L.)	VRS, FT, LTT	New *Fusarium* race, susceptible to anthracnose
Squash (*Cucurbita moschata* Duch.)	VRS, FT, LTT	Inferior fruit shape and quality
Interspecific hybrid squash *(Cucurbita maxima x C. Moschata Duch.)*	VRS, FT, LTT, HTT,SV	Reduced fertilizers required. Some qality reduction may result.
Pumpkin (*Cucurbita pepo*L.)	VRS, FT, LTT	Mostly for cucumber
Wintermelon (*Benincasa hispida* Thunb.)	GDR	Incompatibility
Watermelon [*Citrulluslanatus*(Thunb.) Matsum. Et Nakai]	FT	Not enough vigor and disease resistance
African horned (AH) cucumber (*Cucumis mutiliferus* E. Mey. Ex Naud)	FT, NMT	Medium to poor graft compatibility
Cucumber		
Figleaf gourd (*Cucurbita ficifolia*Bouché)	LTT, GDT	Narrow graft compatibility
Squash (*Cucurbita moschata* Duch.)	FT, FQ	Affected by Phytophthora
Interspecific hybrid squash *(Cucurbita maxima x C. Moschata Duch.)*	FT, LTT	Slight quality reduction expected
Bur cucumber (*Sicyos angulatus* L.)	FT, LTT, SMT, NMT	Reduced yield
AH cucumber (*Cucumis metuliferus* E. Mey. Ex Naud)	FT, NMT	Weak temperature tolerance
Melon		
Squash (*Cucurbita moschata* Duch.)	FT, LTT	Phytophthora infection
Interspecific hybrid squash *(Cucurbita maxima x C. Moschata Duch.)*	FT, LTT; HTT, SMT	Phytophthora infection, poor fruit quality
Pumpkin (*Cucurbita pepo*L.)	FT, LTT and HTT, SMT	Phytophthora infection
Melon (*Cucumis melo* L.)	FT, FQ	Phytophthora problem
AH cucumber (*Cucumis metuliferus* E. Mey. Ex Naud)	FT, LTT, SMT, NMT	Weak temperature tolerance

[a] VRS: Vigorous root systems; FT: *Fusarium* tolerance; LTT: low temperature tolerance; ST: strong vigour; HTT: high temperature tolerance; GDT: good disease tolerance; GDR: good disease resistance; NMT: nematode tolerance; SMT: high soil moisture tolerance; FQ: fruit quality modification.

Table 3 – Rootstocks for solanaceous crop and some major characteristics.

Rootstock	Scion	Major characteristics
S. lycopersicum L.	Tomato	Vigor and virus tolerance
S. lycopersicum L.	Tomato	Nicotine and alkaloid absorption effected
S. lycopersicum L.	Tomato	High temperature tolerance
S. habrochaites S. Knapp & D.M Spooner	Tomato	Resistant to corky root disease
Solanum spp.	Tomato	Resistant to bacterial wilt and nematode; Yield increase
S. sodomaeum L.	Tomato	Growth and yield reduction
S. auricularum L.	Tomato	Growth and yield reduction
*S. lacinatum*Ait.	Tomato	Resistant to water-logging
*S. melongena*L.	Tomato	Growth anf yield reduction
*S. integrifolium*Poir.	Tomato	Sugar content increase
S. sisymbrifolium Lam.	Tomato	Disease resistance, no effect on sugar content
S. torvum Sw.	Tomato, Eggplant	Disease resistance, no effect on sugar content
S. toxicarium Lam.	Tomato	Disease resistance, no effect on sugar content
S. melongena L.	Tomato	Multiple disease resistance
S. nigrum L.	Tomato	Fruit size and quality control
S. lycopersicum L. x *S. habrochaites* S.Knapp& D.M Spooner	Tomato	Low Fusariuminfection
S. lycopersicum L. x *S. habrochaites* S.Knapp& D.M Spooner	Tomato	Multiple disease resistance
S. lycopersicum L.	Tomato	Resistant to corky root (K), Root knot nematode (N), Verticillium wilt (V), and Fusarium wilt (F); Yiels increase
S. melongena L.	Tomato	Low and high temperature tolerance
S. lycopersicum L.	Tomato	Resistant to tomato brown root rot
S. torvum Sw.	Eggplant	Resistant to nematode
S. torvum Sw. x *S. sanitwongsei* Craib.	Eggplant	Resistance to bacterial wilt
S. integrifolium Poir. x *S. melongena* L.	Eggplant	High temperature tolerance
Capsicum spp.	Sweet pepper (green)	Compatible with Capsicum only
*C. annuum*L. X *C. Chinensis* Jacq.	Green pepper	Superior growth and yield
Datura tatula	Tomato	Low yield

1.4 Propagation by grafting in horticulture

Grafting is the art of joining together two pieces of tissue of living plant, so that they develop and grow forming a single plant.

Grafting is a process that involves: (1) the choice of rootstock and scion species, (2) creation of a graft union by physical manipulation, (3) healing of the union, and (4) acclimation of the grafted plant.

Generically, the grafting technique can be practiced by hand or by machine (robotic grafting). In the first case, the grafting and post grafting operations require three to four people, each assigned to a specific step in the process (Lee and Oda, 2003).

Cucurbits are usually grafted once the first true leaf appears but before it reaches full development both in the rootstock and scion seedlings. To reach this stage for both the scion and the rootstock, planting dates will vary depending on the rootstock and scion chosen, greenhouse temperatures, and seed germination criteria. While, solanaceous are usually grafted once that stem diameter allows an easy seedling manipulation. Grafting methods vary depending upon the kind of crops, farmers' experiences and preferences, facilities and machines available, numbers of grafting, and even the purpose of grafting such as grafting for their own uses or for sales only by commercial growers. The requirements for the growth of the rootstock and scion are critical points in the manual and automatic (machine-driver) grafting, but the germination uniformity as well as seedling growth in both rootstock and scion are more critical for the robotic-driven machines. Machine grafting is done using a simple machine or a grafting robot, which is expensive. New machines are currently being developed in Japan and Korea that are much more forgiving and require less labor to operate. The first semiautomatic grafting system for cucumber was commercialized in 1993 and numerous others were developed since then (Richard L. Hassell and Frederic Memmott, 2008). A grafting robot is able to produce 600 grafts per hour with two operators, while with manual grafting is possible making 1000 grafts per person per day (Lee and Oda, 2003; Masanao and Hisaya, 1996; Suzuki *et al*., 1998). However, at present, only in Japan, for watermelon grafted seedlings production, the automated method is enough used (40% of the total watermelon seedlings production) (Lee and Oda, 2003; Masanao and Hisaya, 1996; Suzuki *et al*., 1998).

1.5 Cucurbitaceous grafting methods

1.5.1 Tongue approach grafting

Since the grafting operation would be much more efficient with both scion and rootstock seedlings having similar height, the seeds of scion (usually watermelons, cucumbers, and melons) are sown 5–7 days earlier than the rootstock seeds.

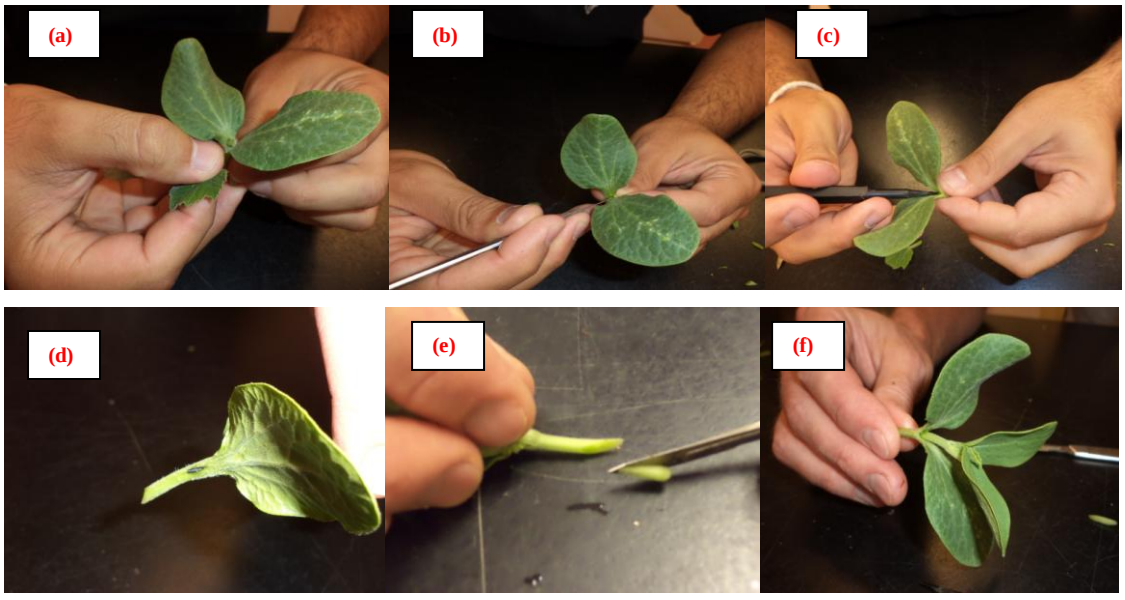
After rootstock shows the fully development of cotyledons, and scion shows the emergence of the first true leaf, is practiced a cut (35° to 45° angle) into the hypocotyl of rootstock approximately halfway with a razor blade, and another cut is practiced an oppositely angled on the hypocotyl of the scion. These cuts need to be made so that the scion will be on top of the rootstock when completed. Two cut hypocotyls are placed together, then sealed with aluminum foil to help healing and prevent the graft from drying out. The grafted plants, are placed into a bigger cell trays (usually 5 cm square x 7.5 cm deep), and then, the trays are watered until soil is completly wet. The grafted plants will be placed into a greenhouse partially shaded for 1 or 2 days before placing them under normal greenhouse growing conditions. The top of the rootstock is cut off 5 d after grafting, and the bottom of the scion is cut off 7 d after the top of the rootstock is removed. After that the bottom of scion is cut off , you must wait 2 days for the plants to be ready to the transplant.

Figure 1 – Tongue approach grafting

1.5.2 Hole insertion grafting

Hole insertion hypocotyl grafting is a method very useful to watermelon grafted seedlings production, because of the smaller seedling size of watermelon as compared to the size of the rootstock (usually squash or bottle gourd). The sowing timeline completely changes in relation to the rootstock used. Watermelon seeds are sown 7–8 days after the sowing of gourd seeds (rootstock) or 3–4 days after sowing squash rootstock seeds. Grafting is made 7–8 days after the sowing of watermelon seeds. The first step is removing the first true leaf and the growing point with a sharp probe (Fig. 2a; 2b), and then open a hole on the upper portion of the rootstock hypocoty (Fig. 2c; 2d) using a wood needle. The scion is then cut on a 35° to 45° angle, both sides, on the hypocotyls (Fig. 2e). The scion is then inserted into the hole made in the rootstock (Fig. 2f). The cut surfaces are matched together and held also without a grafting clip (Fig. 2f). After grafting, the seedlings should be transferred into a humidity chamber. After the healing process, the grafted plants is moved into a greenhouse with a temperature of 20-35 ℃, until the scion is well connected with the rootstock.

Figure 2 – The main steps to make hole insertion grafting

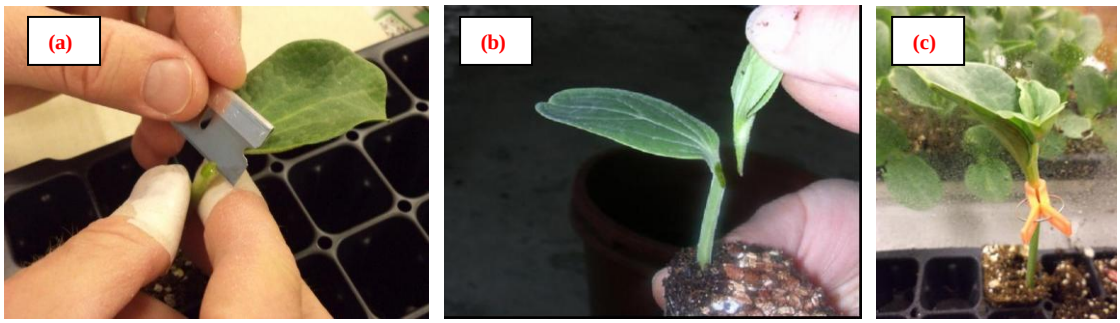


1.5.3 One cotyledon grafting

Seedling production of rootstocks and scions is the same as described for hole insertion grafting. The best stage to perform the grafting correspond to the development of first true leaf of the rootstock plant (7 to 10 days after sowing).

One cotyledon, along with the visible growing point, is cut with a razor blade practing a cut of 35 to 45 angle (Fig. 3a). The hypocotyl of the scion is cut on a 35° to 45° angle (Fig. 3b) on one side only. The two cut surfaces are matched and held together with a grafting clip (Fig. 3b–c). After grafting, the plants should be transferred to a humidity chamber or healing room. After the healing process, the grafted plants is moved into a greenhouse with a temperature of 20-35 °C, until the scion is well connected with the rootstock.

Figure 3 – The main steps to make one cotyledon grafting

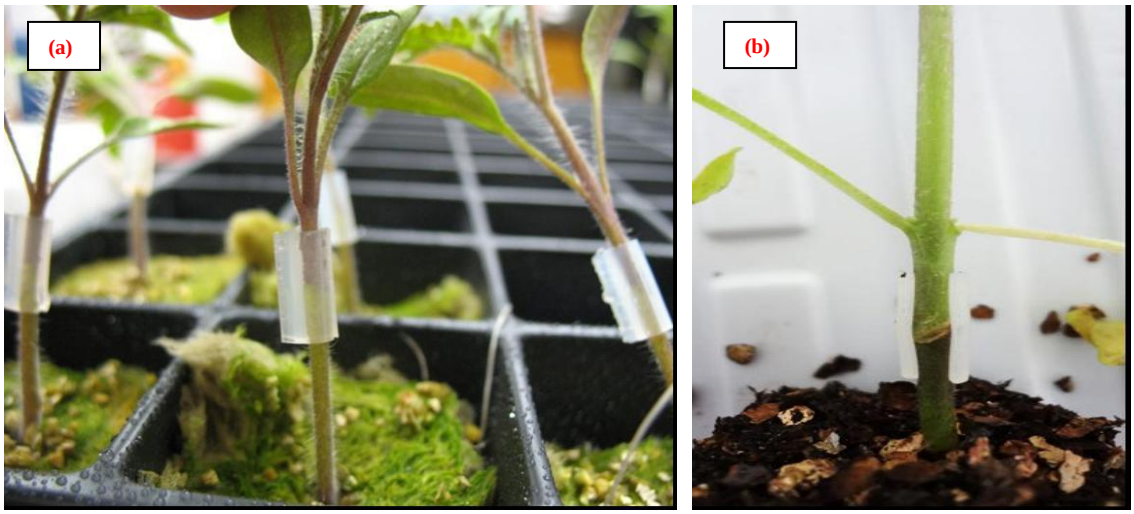


1.6 Solanaceous grafting methods

1.6.1 Tube grafting

Tube grafting method is the more commonly used for solanaceous crops. It is usually performed at lower epicotyl with a cut of about 45° angle both rootstock and scion, and fixed with an elastic silicon tube developed specifically for this type of grafting (Fig. 4a). Tube grafting is completed ensuring a good contact between cut surface of the rootstock and scion. After grafting, the seedlings should be transferred into into a humidity chamber with a temperature of about 30 °C. After the healing process, the grafted plants are moved into a greenhouse with a temperature of 20-35 °C, until the scion is well connected with the rootstock (Fig. 4b).

Figure 4 – The main steps to make tube grafting



1.6.2 Cleft grafting

Cleft grafting was used in cucurbits for a while in several countries, but the use is usually confined to solanaceous crops these days. The rootstock seedlings are sheared with a horizontal cut. A longitudinal cut is performed in a downward direction, 1–1.5 cm long and 3/4 depth of the stem diameter. The scion is pruned to have 1–3 true leaves and the lower part of the stem is cut to obtain a tapered wedge (two cuts of about 45° in opposite sides). After placing the scion into the split made on the rootstock, a clip is placed to hold in position until the union. After grafting, the seedlings should be transferred into a humidity chamber with a temperature of about 30 °C. After the healing process, the grafted plants is moved into a greenhouse with a temperature of 20-35 ℃, until the scion is well connected with the rootstock

Figure 5 – The main steps to make cleft grafting

1.7 Acclimatization

Maintenance of high humidity level (around 100%) during the healing phase is essential for the production of uniform grafted seedlings. To allow an high survival percentage after grafting an adequately acclimatization is fundamental. During this phase occur the healing of the cut surface and the plantlets hardening (Lee and Oda, 2003). Usually, the growers achieve acclimatization by the use of plastic film coverings. In many commercial nurseries, the grafted plants, in cell trays of 32–72 cells, are placed on a greenhouse bench and the trays are sealed with a single layer of semi-transparent high-density polyethylene film (0.01mmor thinner) to reduce the moisture loss and kept sealed for 5–7 days without additional irrigation (Fig.6). During the daytime, partial shading may be necessary to avoid excessive heat build-up.

Figure 6 – Propagation greenhouse during the acclimatization phase

EXPERIMENTAL PART

2
Objectives of research

The researches carried out within the doctoral have various objectives all attributable to the preservation of ecotypes, and their use as scion in order to produce grafted seedlings characterized by better quantitative and qualitative yields. Furthermore, you want to get a significant compression of the nursery propagation time to produce grafted eggplant seedlings, and a reduction of the high transport cost for watermelon grafted seedlings in United States, using the unrooted grafted cutting propagation technique.

Hence, were undertaken the following researches:

Morphological and agronomical characterization of eggplant genetic resources from the Sicily area;

- Grafting suitability of Sicilian eggplant ecotypes onto *Solanum torvum*: fruit composition, production and phenology;
- Agronomical, chemical, histochemical, and histological response of Sicilian eggplant ecotypes to grafting technique;
- Use of unrooted grafted cuttings for watermelon: effect of healing duration and transportation temperature on the cutting and finished plant quality;
- Production of unrooted grafted cuttings of eggplant and field evaluation.

The aim of the thesis consists in the use of grafting as a tool to solve issues related to nursery production, and agronomical performance. All the research activities carried out are focused on the development of new nursery patterns for grafted seedlings production able to overcome national and international issues such as the long time required to produce eggplant grafted seedlings, the low germination percentage of *Solanum torvum* seeds, and the valorisation, through the grafting, of the local genetic resources such as the ecotypes, a countless source of genetic variability.

3

Morphological and Agronomical Characterization of Eggplant Genetic Resources from the Sicily Area

3.1 Introduction

Sicily was an obvious port of call on numerous cultural and trade routes, given its strategic geographical position, and is one of the most interesting centres of origin and differentiation of vegetable crops in the Mediterranean Basin. Over the course of the centuries, farmers have selected various genotypes of each species, adapting them to the soil and climate requirements without paying any particular heed to genetic pureness, but rather allowing the crops to crossbreed spontaneously with wild species in the vicinity. On Sicily alone, over an area of 26,000 km^2, there are an estimated 2,650 taxa, including both specific and intraspecific taxa (Raimondo *et al.*, 1992). This relaxed attitude of the farmers created intraspecific variability leading to genotypes, which were suited to the growth environment, resistant to environmental stress and plant diseases, and with improved qualitative and organoleptic properties (Schiavi *et al.*,1991). Crop improvement to increase productivity has always relied on genetic diversity and, therefore, on the ability of the crop to adapt to soil and climate changes, and it is due to this selection process, used by farmers over the years, that most of the biodiversity has been preserved (Schippmann *et al.* 2002).

The local populations are genotypes of remarkable intrinsic value; their ability to adapt to their original environment could make them more suited to sustainable horticulture than hybrids and varieties created in different soil and climate conditions and which often require higher energy inputs. This is exactly why we consider the aim of this study, that is the rediscovery, recovery, preservation and characterization of local

eggplant (*Solanum melongena* L.) ecotypes, to be crucial, as these ecotypes risk genetic erosion if not adequately safeguarded. The eggplant belongs to the Solanaceae family but, it would seem, its area of origin is not clear. Expert opinion cites the Indies as the centre of origin and China as a secondary centre of diversification 5, only to arrive in Europe around 1300. It was introduced into Italy during the following century, though initially grown only as an ornamental crop and it was only after the 16th century that the fruits were used as food. Today, the eggplant is grown widely throughout the tropical zones and in the temperate regions of the world. The fruit of the eggplant is classified as a non-climacteric berry, which can grow to various sizes, shapes and colours depending on the genotype. Violet is one of the most common colours; the result of anthocyanins in the epicarp, and this colour can be intensified by the presence of chlorophyll pigments in the layers found under the skin (Daunay *et al.*, 2004). The anthocyanins are pigments contained in the cell vacuoles (Daunay *et al.*, 2004; Timberlake, 1981) and belong to the group of phenolic flavonoids (Vinson *et al.*, 1998); compounds found in great number in eggplant berries and known for their antioxidant properties (Cao *et al.*, 1996; Stommel and Whitaker, 2003). Previous studies show that growth environment, cultivation techniques, genotypes and soil type influence the production of these flavonoids (Singh *et al.*, 2009; Todaro *et al.*, 2009) and, as a consequence, anthropic selection has focused on creating black coloured varieties, such as 'Black Beauty', 'Black Campana', 'Mercato Florida', 'Long Black' and others, which are widespread and some of which are still used today (Daunay, 2008; Savin, 1996). There are also many local black eggplant ecotypes (Prohens and Nuez, 2001). The eggplant ecotypes found in Sicily had very dark fruits, such as G2 and G1, but also no colour at all, such as G3.

3.2 Materials and Methods

The field trial was carried out in the open field during the springsummer of 2011 at the experimental fields of the Department of Agri-environmental Systems at Palermo University (38°09'26''N 13°20'01''E). Ecotypes G1, G2, G3, G4, G5 and G6 were evaluated. Ecotypes G2 and G6 were taken from the Marsala area (Trapani), ecotype G1

from Sciacca (Agrigento), ecotype G3 from Bagheria (Palermo), ecotype G4 from Pantelleria (Trapani) and ecotype G5 from Mazara del Vallo (Trapani). In addition, three varieties (Birgah, Black bell and Viola di Firenze) from the National Registry of Varieties were also tested. For each ecotype and variety the randomized block design with three replications of 10 plant blocks were been adopted. Plants and flower observations were taken during the phenological phase, which spanned the period between first inflorescence and pre-harvest. Fruit evaluation was effectuated on the first completely developed fruits. The planting of all the ecotypes took place on 15th May 2011 using seedlings with their root ball. The length of various phenophases was recorded until the end of the production period for every genotype observed. Cultivation took place using growth techniques already in use for open-field eggplant cultivation in Sicily. Seedling bed was prepared through medium-deep ploughing (35 cm) and de-clodding using a rotary harrow. Aged manure was added as a soil amendment at a rate of 40 t ha-1. A drip irrigation system was installed under a 20 μm thick film of black PE.

During the test, a 0.5 m planting distance and a 1 m inter-row distance layout was adopted, thereby obtaining a density of 2 plants/m2. Thirty plants were used for each genotype. A form of free cultivation technique was used and pruning and de-leafing took place only when required. The quantity of fertilizing units used for fertigation was calculated on the basis of nutrient uptake estimation (kg t-1), expected yields and soil mineral content (La Malfa, 1990), and was estimated as the following: 250 kg ha-1 of N, 150 kg ha-1 of P and 250 kg ha-1 of K. Quantitative data on average yields per plant and qualitative data, such as average fruit weight and the average number of fruits per plant, were recorded and correlation analysis was carried out on these last two parameters. Yield, based on the qualitative properties of the berries (D.L. n. 306/2002), was divided into marketable yield and unmarketable yield. Data was also collected on various morphological characteristics, such as plant height, fruit length and maximum fruit diameter, and the ratio between these last two was determined. All the percentage data was subjected to angular transformation ($\hat{O}$= arcsen$(p/100)^{1/2}$) and all the data underwent analysis of the variance. The difference between the averages was evaluated using a significance level of $P \leq 0.05$ (Duncan test).

3.3 Results

The highest total average yield per plant was obtained by ecotype G3, which provided production levels, which were markedly higher, even compared to the varieties from the National Registry of Horticultural varieties included in the test (Birgah, Black bell and Viola di Firenze). Good results were obtained from ecotypes G5 and G6, showing statistically non-significant differences between the two. Ecotypes G2 and G4 were found to be less productive (Table 3.1). Regarding the average marketable yield per plant, good results were obtained from ecotype G1, which, in addition to giving good yield levels, was also found to give the lowest percentage of nonmarketable fruits of all the genotypes in the field. However, G3, G5 and G6 also produced an average unmarketable fruits percentage very close to the varieties in the field. Ecotype G3 did not show any statistically significant difference to that of the variety Birgah, while G5 and G6 produced intermediate levels which fell between Black bell and Viola di Firenze (Table 3.1). The ecotype G2 was found to have the highest average number of marketable fruits per plant. This was followed by G3 but with a statistically significant difference between the two. Ecotypes G4, G5 and G6 produced the lowest levels, though not demonstrating any statistically significant difference between them. As regards the average weight of the marketable fruits, the variety Birgah produced the largest fruits, followed by ecotypes G3 and G5, which did not show any significant difference between the two. The ecotype G1 produced fruits with an average weight which was not found to be statistically different to the varieties Black bell and Viola di Firenze. The fruits with the lowest weight were produced by G2 (Table 3.1). Correlation analysis was conducted on the average fruit yield per plant and the average berry weight. Results of the analysis showed that as the average weight of the fruit increased, the average berry yield per plant decreased. This correlation reached a significance level of 5% (Fig. 3.1). The various ecotypes in this study were found to have several morphological characteristics in common. One of these characteristics was anthocyanin colour of the hypocotyls in the seedling, which was found to differ in intensity between the genotypes in the study (Table 3.2). The anthocyanin colour was also found in the stem of the plant and was present in all the genotypes in the study for entire length of the production cycle (Table 3.3). The habit was also deemed of great interest; a characteristic which undoubtedly influences the microclimate conditions of

the phyllospere. G1, G5 and G6 exhibited a semi-erect habit, ecotypes G3 and G4 exhibited a slightly different growth habit in that they were found to have a prostrate habit, whilst G2 had an erect habit (Table 3.3). The size of the leaf blade was found to be small in ecotypes G2 and G4, medium sized in G5, G3 and G1 but large in G6. The degree of sinuation of the leaf margin varied from strong in ecotype G4, to absent in ecotype G2. A common characteristic among the 5 ecotypes was the weak leaf undulation, except in G1, which was found to have medium leaf undulation of the leaf blades (Table 3.3). As regards the flowers, the only ecotype found to have a multiflowered inflorescence was G2, with an inflorescence of small, dark purple flowers (Table 3.4). Concerning the main characteristics of the berry, the berry shape was the initial aspect under evaluation, and the genotypes in the study showed marked differences. Only 2 of the 6 ecotypes produced fruits with the same shape, that is G3 and G5, which had an ovoid shape. G6 produced globular fruits, G4 produced obovate fruits, G1 produced pear-shaped fruits and ecotype G2 produced cylindrical fruits (Table 3.5). The main skin colour of 5 of the genotypes was violet, whereas ecotype G3 was white in skin colour. The fruits of G5 and G6 were found to have stripes and G6 also had a degree of mottling. Ecotype G1 was of particular interest in that its skin was remarkably dark with a high level of glossiness. One characteristic of G2 was the greenish colour of the flesh (Table 3.6). The tallest plants were found in the two varieties Birgah and *Black bell*, followed by ecotype G2. Ecotype G3 was found to produce the shortest plants; however, no statistically significant differences were found with G1 and G6 (Table 3.7). Other data collected concerned the shape index, in particular the ratio between the maximum diameter and the length. The highest values for this ratio were found in ecotype G2; explained by the fact that it was cylindrical in shape, whilst the lowest value was found in G6, the only globular fruit, although this value did not show any significant difference to ecotype G5 and the variety
Black bell (Table 3.7). The phenological phase of flowering began with ecotype G2, which took place at the end of the first ten days of June. The other ecotypes began flowering 10 days afterwards. This earliness was also reflected in the production cycle, which finished approx. 7 days earlier than the other biotypes (Fig. 3.2).

3.4 Conclusions

The production of high quality vegetables is often linked to the use of local ecotypes (Piergiovanni and Laghetti, 1999; Rao *et al.,* 2006). These ecotypes are subjected to low environmental impact agricultural techniques, typical of the areas from which they are selected, and this allows qualitatively high levels to be reached, often higher than registered varieties. Indeed, the prolonged use by farmers of genotypes selected from different soil and climate conditions can even lead to financial loss (Babcock and Clemens, 2004). Of the ecotypes, G3 and G1 reached production levels which were markedly superior to the varieties. Quality characteristics, such as unmarketable fruits, skin colour, glossiness and flesh colour, produced by the ecotype G1 were highly sought after on the local market. Therefore, the recovery, characterisation and diffusion of old, native populations is not the start of agricultural and cultural regression but rather the chance to help face ecological issues and those concerning agro ecosystem sustainability. In conclusion, the results of this study provide new knowledge on the agronomic behaviour of several accessions. Local ecotypes could happily be taken into consideration as part of new crop systems as they clearly reached a certain level of production efficiency and an average fruit weight similar to, or even higher than, those of the traditional varieties used as a reference. The high average fruit weights, of biotypes G3 and G1 in particular, would increase the labour productivity for harvesting. Furthermore, the use of these genotypes would produce berries with the added value of 'being typical'; a characteristic which is very important when attempting to add economic value to a product.

Table 3.1 – Production traits of the eggplant ecotypes and varieties tested

Genotype	Average total production/plant [kg]	Average marketable /plant [kg]	Average number marketable fruits/plant [n]	Average Weight marketable Fruits [g]	Average unmarketable Fruit Production/plant [%]	Average number unmarketable Fruit/plant [%]
G1	3.8 b	3.5 b	8.51 bc	409.05 c	7.77 g	11.69 d
G2	2.9 d	2.4 f	15.69 a	156.03 e	17.32 bc	20.83 c
G3	5.8 a	4.9 a	9.01 b	548.00 b	14.97d	20.69 c
G4	2.4 e	1.8 g	5.17 d	350.79 d	24.39 a	34.76 a
G5	3.3 c	2.9 de	5.50 d	526.83 b	12.89 e	25.78 bc
G6	3.2 c	2.7 e	5.13 d	528.59 b	16.33 cd	24.01 bc
Birgah	3.4 c	2.9 d	3.87 d	762.94 a	13.87 d	29.80 ab
Black bell	3.3 c	3.0 d	7.30 c	409.81 c	10.50 f	18.29 c
Viola di Firenze	3.9 b	3.2 c	7.60 bc	422.04 c	18.86 b	23.74 bc

In each column, figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 3.2 – Main characteristics of seedlings of the 6 eggplant ecotypes

Ecotype	Anthocyanin colour of hypocotyls	Intensity of anthocyanin colour of hypocotyls
G1	present	very weak
G2	present	weak
G3	present	weak
G4	present	weak
G5	present	strong
G6	present	strong

Table 3.3 – Main characteristics of plant, stem and leaves of the six eggplant ecotypes

	Plant	Stem			Leaves		
Ecotypes	Habit	Anthocyanin colouration	Intensity of Anthocyanin colouration	Pubescence	Size of leaf blade	Sinuation of margin	Leaf undulation
G1	Semi-erect	present	very weak	medium	medium	medium	medium
G2	erect	present	medium	weak	small	absent or very weak	absent or very weak
G3	prostrate	present	very weak	medium	medium	weak	weak
G4	prostrate	present	very weak	weak	small	strong	absent or very weak
G5	Semi-erect	present	medium	medium	medium	medium	weak
G6	Semi-erect	present	strong	medium	large	medium	weak

Table 3.4 – Main characteristics of the flowers of the six eggplant ecotypes

Ecotypes	no. flowers/inflorescence	Size	Intensity of purple colour
G1	1	medium	light
G2	More than 3	small	dark
G3	1	medium	light
G4	1	medium	light
G5	1	medium	light
G6	1	medium	medium

Table 3.5 – Main morphological characteristics of the fruits of the six eggplant ecotypes

Ecotypes	Shape	Ribbing	Apex of fruit	Size of calyx	Length of stalk
G1	Pear-shaped	weak	indented	medium	medium
G2	cylindrical	absent or very weak	rounded	medium	medium
G3	ovoid	weak	rounded	large	medium
G4	obovate	absent or very weak	indented	large	short
G5	ovoid	medium	rounded	medium	medium
G6	globular	medium	rounded	large	medium

Table 3.6 – Main characteristics of skin and flesh colouration of the 6 eggplant ecotypes

Ecotypes	Main colour of skin	Intensity of main colour	Glossiness	Mottling	Striping	Importance of striping	Density of striping	Colour of flesh
G1	violet	very dark	strong	absent	absent	-	-	whitish
G2	violet	very dark	medium	absent	absent	-	-	greenish
G3	white	-	weak	absent	absent	-	-	whitish
G4	violet	medium	medium	absent	absent	-	-	whitish
G5	violet	light	medium	absent	present	medium	medium	whitish
G6	violet	medium	weak	present	present	medium	medium	whitish

Table 3.7 – Main size characteristics of the plant and fruit of the 6 eggplant ecotypes

Ecotypes	Plant height [cm]	Fruit length [cm]	Fruit diam. [cm]	length/diam.
G1	48.33 d	15.50 c	11.83 c	1.31 c
G2	66.33 b	27.00 a	6.05 d	4.48 a
G3	48.11 d	14.50 c	12.42 b	1.17 c
G4	60.44 c	17.67 b	11.00 c	1.66 b
G5	64.67 bc	15.33 c	14.50 ab	1.06 cd
G6	52.56 d	12.53 d	11.89 c	1.05 cd
Birgah	77.78 a	11.67 d	15.67 a	0.74 e
Black bell	78.55 a	15.5 c	14.50 ab	1.08 cd
Viola di Firenze	61.33 bc	12.00 d	14.65 ab	0.82 d

In each column. figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Figure 3.1 – Relationship between the average number of fruits per plant and the average marketable fruit weight

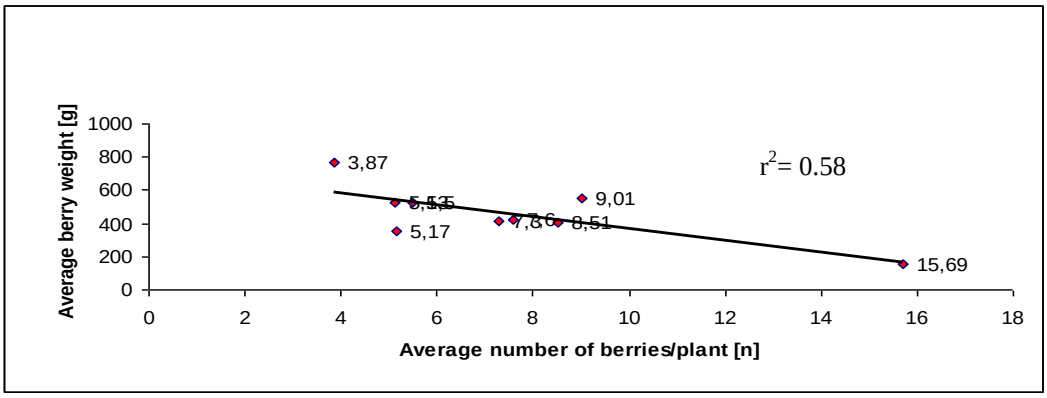
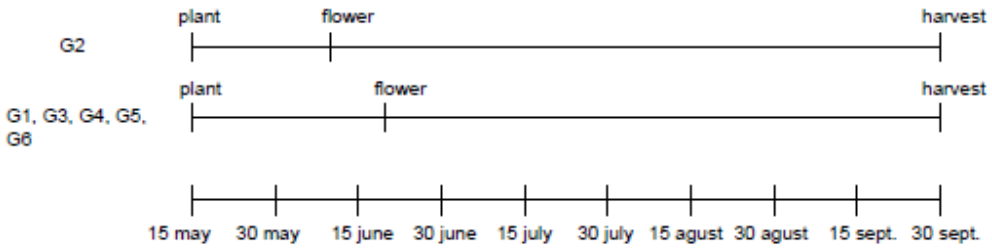


Figure. 3.2 – Phenogram of the 6 eggplant ecotypes



4

Grafting Suitability of Sicilian Eggplant Ecotypes onto Solanum torvum: Fruit Composition, Production and Phenology

4.1 Introduction

Vegetables in general are a great source of minerals in the human diet (Cao *et al.*, 1996; Rubatzky and Yamaguchi, 1997) and the eggplant (*Solanum melongena* L.) provides significant quantities of various minerals, amongst which P, K, Ca and Mg (Flick *et al.*, 1978; Savvas and Lenz, 1996). The quantities in which they are found is highly dependant upon cultivation technique (Hanson et al., 2006; Russo, 1996; Savvas and Lenz, 1996). It has been shown that fertigation effects the mineral composition of the fruit (Russo, 1996), as does the saline level of the water used in cultivation (Savvas and Lenz , 1996).

The eggplant (*Solanum melongena* L.) is one of the most widely cultivated crops in tropical and temperate regions around the world and is suitable for propagation through grafting (Bletsos *et al.*, 2003; Daunay, 2008). A lack of genetic material tolerant or resistant to abiotic or biotic telluric stress together with a ban on soil sterilization using methyl bromide has led to increased interest worldwide in the technique of grafting in vegetable species (Bletsos, 2005; Davis *et al.*, 2008a b King *et al.*, 2008 ; Miguel *et al.*, 2004.

Solanum torvum Sw. is considered to be one of the most suitable rootstocks for the eggplant, providing resistance to a large number of telluric pathogens (*Verticillium dahliae* Klebahn, *Ralstonia solanacearum* (Smith) Yabuuchi *et al.*, *Fusarium oxysporum* (Schlechtend:Fr.) f. sp. *melongenae* Matuo and Ishigami, and *Meloidogyne*

spp. root-knot nematodes, *Ralstonia solanacearum*) (Bletsos *et al.*,2003; Daunay, 2008; Singh and Gopalakrishnan, 1997; King *et al.*, 2010).

As mentioned above, it is possible to cultivate high eggplant quality genetic material, such as local populations, even if susceptible to telluric pathogens, by grafting onto tolerant or resistant rootstock (Colla *et al.*, 2010), such as *Solanum torvum* Sw..

The use of ecotypes has always been dependant upon their ability to adapt to the environment of origin thanks to their distinct genetic characteristics.

The selection process, used by farmers over the years in order to increase yields or other useful characteristics by using mass selection and without any attempt to control fertilization, has led to the preservation of a large part of the biodiversity (Schippmann *et al.*, 2002). This has also preserved intraspecific variability, providing genotypes which are suited to the growth environment and possibly resistant to environmental stress, plant diseases and with improved qualitative and organoleptic properties (Schiavi *et al.*, 1991).

An important aspect, which is often overlooked, concerning the technique of grafting, relates to differences in the quality of the fruits produced from grafted or non-grafted plants (Davis *et al*., 2008a), that frequently change due to genotype (Moncada *et al.*, 2013). In the case of ecotypes, as they are high-quality genetic material, this aspect is of even greater importance.

Some studies report positive effects on the quality of the fruit from grafting, such as with mini-watermelons (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) from plants grafted onto intraspecific hybrids of *Cucurbita moschata* Poir. x *Cucurbita maxima* Duch, which showed higher levels of K, Mg, lycopene and vitamin C compared to the control group (Proietti *et al.*, 2008).

However, negative effects were obtained from grafting tomato plants onto *Solanum integrifolium*, which gave an extremely high incidence of fruits affected by rot (Oda *et al.*, 1996). In the case of eggplant grafted, for example, onto *Datura inoxia* P. Mill., grafting caused levels of atropine and scopolamine which were high enough to cause poisoning (Oshiro *et al.*, 2008).

The aim of this study is to look at aspects regarding the quality of the fruit, production and phenology of four Sicilian eggplant ecotypes grafted onto *Solanum torvum* Sw..

Ecotypes that are distinguished by their morphological and agronomical characteristics are very different (D'Anna and Sabatino, 2013).

4.2 Materials and methods

4.2.1 Plant material

The plant material used in this study comprised 4 ecotypes found in Sicily in the provinces of Palermo, Trapani and Agrigento, which exhibited very different morphological characteristics (Table 1).

Solanum torvum Sw. Australys selection, (Agri Seeds s.r.l.) was used as a rootstock.

For the production of plant material for the tests, on the 20th February 2011 seeds for the rootstock (*S. torvum* Sw.) were planted in 44-cell seedling trays at a density of one seed per cell in order to calculate the germination rate, under a temperature regime of 25 °C/18 °C (day/night) in a propagation greenhouse. After 20 days, seeds from the 4 ecotypes were planted in 100-cell trays and given the same temperature regime and planting method as the rootstock.

75 days after planting the *S. torvum* Sw., both the rootstock and scion had reached an adequate diameter to allow for grafting. The grafting involved cutting off the rootstock at a 45° angle and making a similar cut on the scion. Care was taken to make sure that the diameters of the rootstock/scion were nearly identical so that the two exchange sites fitted perfectly. Grafting was completed by attaching a clip to ensure the correct fit and the correct amount of pressure was applied. The grafted plants were kept at a temperature of 20°C and a humidity rate of 95% for 10 days in order to encourage histological processes.

The substrate used contained peat moss Thechinic (DUEEMME marketing s.r.l.).

4.2.2 Setting up and managing the test trial area

The test trials for the evaluation of grafting on the ecotypes in the study started on 15th May 2011 at the experimental fields of the Department Agricultural Science and Forestry in Palermo.

For all of the ecotypes, both grafted and non-grafted, 3 replication of 10 plants were used in a randomized block design. The plants were placed in soils classified as Alfisols "Red Mediterraenan soils".

A good seedling bed was prepared by carrying out medium-depth ploughing (35cm) and de-clodding of soil clumps using a rotary harrow. Aged manure was added as a soil amendment at a rate of 40 $t*ha^{-1}$. A drip irrigation system was installed under a 20 μm black PE film.

A planting distance of 0.5 m and an inter-row distance of 1 m were adopted, thereby obtaining a density of 2 plants/m^2. A type of free cultivation technique was used, and pruning and de-leafing took place only when required. On the grafted plants, the shoots coming from the rootstock were eliminated.

The number of fertilizing units used for fertigation was calculated on the basis of hypothetical uptake ($Kg*t^{-1}$), expected yields and soil mineral content (La Malfa, 1990) , and was the following: 250 $Kg*ha^{-1}$ of N, 150 $Kg*ha^{-1}$ of P_2O_5 and 250 $Kg*ha^{-1}$ of K_2O.

Six manual harvests were carried out as soon as the berries reached commercial ripening.

4.2.3 Morphological, production and phenological data collection

Morphological data were collected on plant height 35 and 60 days from planting, and leaf number 35 days after planting. Production data included average total yield/plant, average marketable yield/plant, average number of marketable fruits/plant, average marketable fruit weight and percentage of discarded fruits.

The length of the different phenophases was also recorded.

4.2.4 Sampling

Sampling for the quality analysis of the fruits was carried out using 3-5 commercially ripe fruits for each replication from the 2^{nd} and 3^{rd} harvests; only healthy fruits were chosen. Care was taken to ensure that each sample contained the same percentage weight of apical, middle and distal parts of the fruit.

4.2.5 Quantitative analysis of the fruit

The water content was determined in a ponderable way and the ash content was determined on a 5 g sample rate. The eggplant sample was weighed in a platinum capsule, calibrated at 550 °C and heated to 150 °C for 6-8 h. The sample was

subsequently incinerated on a flame and then in a muffle furnace at 550 °C for 6-8 h. The ash content was obtained by quantitative determination of the residual product. The water content was obtained, in ponderable way, through the dehydration of the sample, in the presence of sand, in a heater at 105 °C per 6-8 h. Protein determination was obtained following the Kjeldal method. A sample rate was subjected to acid-catalizated mineralization in order to turn the organic nitrogen into ammoniacal nitrogen, which was then distilled in an alkaline pH. The ammonia formed during this distillation was collected in a boric acid solution and determined through titrimetic dosage. The value of ammoniacal nitrogen was multiplied by 6.25.

The ash, water and protein contents were analysed according to standard official analytical methods. Ca, Mg, Na, K, Fe, Mn, Z and Cu were determined using atomic absorption spectroscopy following wet mineralization according to Morand e Gullo methodology (Morand e Gullo,1970). Phosphorous levels were determined using colorimetry based on FOGG D.N. methodology (FOGG.D.N., WILKINSON N.T.,1958).

4.2.6 Statistical analysis

Data for each of the characteristics being evaluated were subjected to one-way analysis of the variance (ANOVA). Before each elaboration, all the percentage values were subjected to angular transformation ($\Phi = \text{arcsen}(p/100)^{1/2}$).

Furthermore, in order to evaluate the average effect of the grafting on the characteristics recorded, orthogonal comparisons were made between the ecotypes which were grafted *versus* not-grafted. Linear correlations were also carried out to perform pair wise comparisons of some detected character.

4.3 Results and discussion

4.3.1 Seed germination and graft-take

All the ecotypes reached a germination rate of over 95% (Table 4.2), whereas the average results for *S. torvum* were found to be around 85%. Graft-take was 100% for all ecotypes.

4.3.2 Morphological, production and phenological data

Grafting in eggplants is a propagation method which aims to increase water and nutrient uptake to the plant (Rouphael *et al.,* 2008). In our case, 35 days after planting the grafted plants were more developed; in particular grafted ecotype B4 was found to have a statistically greater height than all the others. However, it is also worth noting that the same observation made after 60 days gave differences that were not found to be statistically different between the different propagation techniques, although B4 continued to have the greater height. Significant differences were found regarding leaf number 35 days after planting; ecotype B4, both grafted and non-grafted, was found to be the greatest for this characteristic, too. The lowest leaf number at this stage of the biological cycle was found for ecotypes B2 and B3, both grafted and non-grafted, compared to ecotype B4 grafted and not. Grafting did not influence this characteristic (Table 4.3).

As regards productivity, the most productive ecotype was B1 which, both grafted and non-grafted, gave significantly higher yields than B4 grafted and non-grafted (which gave the lowest yields). The ecotypes behaved in the same way for both total yield and marketable yield. Grafting significantly increased marketable yield but it did not affect the percentage of discarded fruit.

The largest average number of fruits per plant was produced by the ecotype B4 both grafted and non-grafted, whilst statistically significant differences were not found between the other ecotypes. It is also worth noting the fact that, concerning all the abovementioned production characteristics, grafting was found to be a useful technique for increasing the production potential (Colla *et al*, 2006).

As regards the average fruit weight, ecotype B4, both grafted and non-grafted, produced fruits which weighed the least.

Excellent results were obtained with ecotype B2 concerning the percentage of discarded fruits, although a comparison of both grafted and non-grafted ecotypes did not produce any significant differences. This shows that the greatest production potential of the ecotypes in the study was given by a greater number of marketable fruits, meaning also that the increment of production potential mainly concerned the marketable fruits (Table 4.4).

Data on the dry berry matter and that of the plant at the end of the production cycle (Table 4.6) were of great interest. The dry berry matter produced by the grafted plants was higher whilst the dry plant matter of the grafted plants at the end of the production cycle was lower, even though the grafted plants were taller 35 days after planting. The explanation for this may be due to the fact that the greater nutrient and water uptake by the grafted plants during the initial stage of the biological cycle was used for the vegetative activity of the plant, whereas, when started the reproductive phase, nutrients and water were channelled towards the strongest sink – the fruit. This hypothesis seems confirmed by the fact that production on the grafted plants was higher and the plant, at the end of the production cycle, was more exhausted and had a lower level of dry matter compared to the non-grafted plants, at least under the growth conditions of this study. Furthermore, ecotype B4 reached the flowering stage approx. 7 days before the others (Table 4.5). It is worth noting that grafting did not determine any changes in the length of the various phenophases.

4.3.3 Qualitative analysis of the fruit

Based on analysis carried out regarding the metal content in the fruit, grafting did, indeed, seem to have an effect both on the macroelement content and the microelement content (Tab.4.6).

This is in agreement with previous studies which claim that grafting can affect the quality of the final product (Lee e Oda, 2003). Grafting onto *Solanum torvum* was found to lead to an increase in the protein content and some of the macronutrients, such as K and Ca, but also microelements, such as Fe, Zn and Cu. Furthermore, evaluation of the berries from the grafted plants showed lower levels of Na, P, Mg and Mn compared to the fruits from the plants propagated by seed.

Of the ecotypes in the study, ecotype B2 was found to have the highest protein levels.

As regards Ca, ecotype B4 when grafted produced the highest levels, showing that this technique greatly affects the content of this element in the berries. The P content, however, in grafted plants was found to be lower, although the differences with fruits from seed propagated plants were not found to be significantly different. In any case, the ratio Ca/P for all of the ecotypes was found to be well below 1, which is the value considered to be optimal for a good uptake of both of the metals (McDowell, 2003).

A marked difference concerning the micronutrient Cu content was found between fruit from the grafted and the non-grafted plants. The highest content was found in ecotype B4 with an increase of 63.1% in the same ecotype which had been grafted. This is confirmed by other authors who evaluated the presence of metals in the berries of eggplant varieties with purple, green and white epicarps (Flick *et al.*,1978).

The ash content was also affected by the propagation method, found to be slightly higher in the berries of the grafted plants.

The soluble solids content was found to be highest in ecotype B3 non-grafted, although grafting was not found to affect this parameter (Table 4.6 bis).

The results of this study would seem to agree with literature where, in some cases, fruit quality is negatively affected by grafting (Lee, 1994; Nisini *et al.*, 2002) and, in other cases, it improves the characteristics of the fruit (Bletsos *et al.*, 2003).

4.3.4 Correlations

Correlations between plant height 35 days from planting and production data (Table 4.7), in the case of non-grafted plants, were found to be negative for total average yield per plant, average marketable yield per plant and average weight of marketable fruits. Positive correlations were found, however, between plant height after 35 days and the number of marketable fruits, as were found between plant height after 35 days and the percentage of discarded fruits.

This means that, in the non-grafted plants, greater plant vigour is to the detriment of yields, which were found to be lower.

The plants propagated using grafting behaved very differently; plant height after 35 days was positively correlated only with the number of marketable fruits produced on average per plant and the percentage of discarded yield. This means that greater plant height in this production phase leads to greater production potential, although in part due to an increase in the production of non-marketable fruits.

Other correlations concerned the dry plant matter at the end of the production cycle and production data (Table 4.7). From these analyses, the non-grafted dry plant matter resulted as being negatively correlated to the average production of marketable fruit per plant and positively correlated to the percentage of discarded fruits.

We might deduce from these results that when seed propagated plants accumulate nutrients in the vegetative parts, a smaller quantity of marketable fruits are produced, due to an increase in the discarded fruit production.

Two negative correlations were found on the grafted plants between the dry plant matter at the end of the production cycle and both the average total yield per plant and the average marketable yield per plant. This may be explained if we refer back to the source-sink theory, or rather, that within the plant there is a hierarchy for the translocation of assimilates as follows (Wardlaw, 1990):

Seeds > fleshy parts of fruit = apical shoots and leaves > cambium > roots > reserves

Therefore, the greater production potential of the grafted plants, in this case, determine an impoverishing effect on the plant.

Regarding the correlations proteins/metals and proteins/ash (Table 4.8), fruits from non-grafted plants did not produce any significant correlations, whereas fruits from grafted plants produced two negative correlations between protein and P, and protein and Fe. As far as fruit yields from non-grafted plants are concerned, correlation analysis between dry berry matter (DM berries) and metals, and dry berry matter and ash produced a positive correlation between DM berries and K, whereas, as regards fruits from grafted plants, DM berries was positively correlated to Ca, Na, Fe and Cu, and negatively to Zn.

4.4 Conclusions

Results from this study highlighted the importance of grafting in the exploitation of local eggplant populations. Genetic material from these populations, as it is, although having excellent organoleptic qualities, does not represent a realistic alternative to the hybrids used commercially today. In addition, the research showed that some metals, such as Na and Mn, fell in level in the fruits from grafted plants. This variation could be of significant interest as lower levels favour a reduction in hypertension and help keep blood pressure under control.

Ecotypes in the study may represent a good source of biodiversity for breeding programmes for the improvement of fruit quality.

In conclusion, this research confirmed that the rootstock used gives the ecotypes in the study greater vigour in the initial 35 days from planting, an increase in production due to the greater number of marketable fruits and more wholesome fruit.

Table 4.1 – Origin and brief morphological description of the ecotypes in the study

Ecotypes	Geographical Coordinates	Shape of fruit	Colour of fruit	Intensity of fruit colour	Ribbing	Blotching
B1	38°4’49’’44N 38°30’36’’72 E	ovoid	white	-	weak	absent
B2	37°30’33’48N 13°5’20’’04E	pear-shaped	purple	very dark	weak	absent
B3	37°49’20’’28N 12°29’28’’80E	globular	purple	medium	medium	present
B4	37°39’54’’72N 12°35’20’’04E	cylindrical	purple	very dark	absent	absent

Table 4.2 – Percentage of seed germination in 4 eggplant ecotypes

Ecotypes	% germination
B1	98 ns
B2	99.5 ns
B3	97.67 ns
B4	98.33 ns

Figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 4.3 – Morphological data on 4 eggplant ecotypes grafted and non-grafted

	h 35 days	h 60 days	leaf no. at 35 days
B1 grafted	62.80 abc	107.53 ns	38.37 bc
B1 non grafted	58.87 abc	109.53 ns	41.00 abc
B2 grafted	58.00 abc	110.40 ns	29.40 c
B2 non grafted	56.67 bc	101.47 ns	29.73 c
B3 grafted	61.13 abc	106.87 ns	30.53 c
B3 non grafted	56.60 c	105.73 ns	34.67 c
B4 grafted	67.07 a	115.27 ns	53.47 a
B4 non grafted	65.50 ab	108.40 ns	51.30 ab
grafted vs non grafted	*	ns	ns

In each column, figures followed by the same letter are not statistically different, based on the Duncan test ($P \leq 0.05$). Significance of orthogonal comparison between grafted *vs* non grafted accessions is reported.

Table 4.4 – Production data on 4 eggplant ecotypes grafted and non-grafted

	Average total production/plant [Kg]	Average marketable production/plant [Kg]	Average number marketable fruits/plant [n]	Average Weight marketable Fruits [g]	Discarded production [%]
B1 grafted	5.18 a	4.38 a	7.61 b	684.33 a	15.50 b
B1 non grafted	4.26 ab	3.62 ab	6.51 b	667.67 a	14.93 b
B2 grafted	4.12 abc	3.79 ab	8.57 b	543.00 a	8.10 c
B2 non grafted	3.77 abc	3.48 abc	6.96 b	487.00 a	7.67 c
B3 grafted	3.38 bcd	2.81 bcd	5.64 b	639.00 a	16.93 ab
B3 non grafted	3.06 bcd	2.57 bcd	4.79 b	608.33 a	16.03 ab
B4 grafted	2.77 cd	2.27 cd	16.34 a	170.33 b	18.00 a
B4 non grafted	2.23 d	1.82 c	14.00 a	160.67 b	18.37 a
grafted vs non grafted	*	*	*	ns	ns

In each column, figures followed by the same letter are not statistically different, based on the Duncan test ($P \leq 0.05$).
Significance of orthogonal comparison between grafted *vs* non grafted accessions is reported

Table 4.5 – Phenogram of 4 eggplant ecotypes grafted and non-grafted

	Date plant	Date flower	Date harvest
B1 grafted	05-15-2011	06-20-2011	09-30-2011
B1 non grafted	05-15-2011	06-20-2011	09-30-2011
B2 grafted	05-15-2011	06-20-2011	09-30-2011
B2 non grafted	05-15-2011	06-20-2011	09-30-2011
B3 grafted	05-15-2011	06-20-2011	09-30-2011
B3 non grafted	05-15-2011	06-20-2011	09-30-2011
B4 grafted	05-15-2011	06-13-2011	09-30-2011
B4 non grafted	05-15-2011	06-13-2011	09-30-2011

Table 4.6 – Composition and mineral content of 4 eggplant ecotypes grafted and non-grafted

	Proteins [g/100g]	K [mg/100g]	P [mg/100g]	Ca [mg/100g]	Na [mg/100g]	Mg [mg/100g]	Fe [ppm]	Zn [ppm]	Cu [ppm]	Mn [ppm]	Ceneri [g/100g]
B1 grafted	13.33 ab	307.38 ab	340.59 b	107.04 b	71.21 bc	16.84 b	38.17 a	40.33 b	3.70 ab	4.79 ab	8.43 a
B1 non grafted	12.71 ab	341.47 ab	387.06 ab	103.72 b	78.14 ab	19.70 b	29.20 c	32.93 c	2.26 b	7.03 a	7.95 ab
B2 grafted	13.73 a	332.14 ab	369.41 b	105.17 b	74.73 abc	16.54 b	30.80 bc	40.83 b	3.83 ab	5.93 ab	8.27 ab
B2 non grafted	13.52 a	350.71 a	392.43 a	105.14 b	81.35 ab	18.64 b	28.97 c	39.60 bc	2.33 b	6.00 a	7.94 ab
B3 grafted	12.46 b	291.43 b	366.72 b	101.26 b	64.39 c	19.05 b	36.63 ab	48.37 a	2.60 b	2.60 b	7.12 ab
B3 non grafted	12.17 b	321.52 ab	391.49 a	100.43 b	65.92 c	19.69 b	35.77 ab	39.73 bc	2.00 b	5.33 ab	7.01 b
B4 grafted	12.54 b	305.63 ab	359.67 b	143.06 a	80.09 ab	16.69 b	38.23 a	40.50 b	5.23 a	3.30 ab	8.04 ab
B4 non grafted	12.15 b	346.47 ab	378.00 ab	103.42 b	84.38 a	25.70 a	34.93 abc	35.60 bc	3.30 ab	5.30 ab	7.81 ab
grafted vs non grafted	*	ns	ns	***	**	**	***	**	*	**	ns

In each column, figures followed by the same letter are not statistically different, based on the Duncan test ($P \leq 0.05$). Significance of orthogonal comparison between grafted *vs* non grafted accessions is reported

Table 4.6 bis – Composition and mineral content of 4 eggplant ecotypes grafted and non-grafted

	Soluble solids	DM berries	DM plant
	[Brix°]	[%]	[%]
B1 grafted	4.46 bcd	6.21 b	2.62 bcd
B1 non grafted	4.01 d	4.99 e	3.41 ab
B2 grafted	4.10 cd	7.81 a	2.31 d
B2 non grafted	4.27 bcd	5.41 d	2.57 cd
B3 grafted	4.82 bc	5.66 cd	3.21 abc
B3 non grafted	5.83 a	5.50 d	3.47 a
B4 grafted	4.93 b	7.65 a	3.34 abc
B4 non grafted	4.11 cd	6.10 bc	3.85 a
grafted vs non grafted	ns	*	***

In each column, figures followed by the same letter are not statistically different, based on the Duncan test ($P \leq 0.05$). Significance of orthogonal comparison between grafted *vs* non grafted accessions is report

Table 4.7 – Linear correlations between morpho-physiological characteristics and production characteristics

		Average total production/plant	Average marketable production/plant	Average number marketable fruits/plant	Average Weight marketable Fruits	Discarded production
Non grafted	h 35 days	-0.528*	-0.558*	0.829 **	-0.815**	0.510 *
Grafted		-0.298 n.s.	-0.374 n.s.	0.607 *	-0.462 n.s.	0.583 *
Non grafted	DM plant	-0.444 n.s.	-0.531 *	0.211 n.s.	-0.280 n.s.	0.755 **
Grafted		-0.671 *	-0.670 *	0.080 n.s.	-0.251 n.s.	0.283 n.s.

ns, *, **, *** indicate non-significant, or significant at P = 0.05, = 0.01 and = 0.001, respective

Table 4.8 – Linear correlations between proteins and dry berry matter with metals and ash

		Proteins	P	K	Ca	Na	Mg	Fe	Zn	Cu	Mn	Ash
Non grafted	Proteins		0.401 $^{n.s.}$	0.001 $^{n.s.}$	0.370 $^{n.s.}$	-0.006 $^{n.s.}$	-0.38 $^{n.s.}$	-0.270 $^{n-s.}$	-0.394 $^{n.s.}$	-0.234 $^{n.s.}$	-0.203 $^{n.s.}$	0.495 $^{n.s.}$
Grafted			-0.600*	0.340 $^{n.s.}$	-0.285 $^{n.s.}$	0.107 $^{n.s.}$	-0.031 $^{n.s.}$	-0.520*	-0.228 $^{n.s.}$	-0.335 $^{n.s.}$	0.379 $^{n.s.}$	0.399 $^{n.s.}$
Non grafted	DM. berries	0.408 $^{n.s.}$	0.215 $^{n.s.}$	0.657 *	0.06 $^{n.s.}$	0.,384 $^{n.s.}$	-0.333 $^{n.s.}$	-0.270 $^{n.s.}$	0.493 n.s.	-0.347 $^{n.s.}$	-0.180 $^{n.s.}$	0.227 $^{n.s.}$
Grafted		-0.344 $^{n.s.}$	-0.263 $^{n.s.}$	0.426 $^{n.s.}$	0.920**	0.579*	-0.434 $^{n.s.}$	0.569*	-0.657 *	0.593*	-0.312 $^{n.s.}$	0.259 $^{n.s.}$

ns, *, **, *** indicate non-significant, or significant at P = 0.05, = 0.01 and = 0.001, respectively

5

Agronomical, Chemical, Histochemical, and Histological Response of Sicilian Eggplant Ecotypes to Grafting Technique

5.1 Introduction

Sicily is the largest Mediterranean island located in southern Italy. It is a cultural and a commercial port, and one of the most important centre of origin and differentiation of vegetables (D'Anna and Sabatino, 2013). During the centuries, the farmers obtained many genotypes for each species, adapting them to the pedoclimatic requirements, and don't caring them to the genetic purity. For this reason, it was estimated a presence of 2650 taxa (Raimondo *et al.,* 1992) in Sicily on an extension of 26000 Km^2. This selection criteria allowed to obtain an inter-specific variability that brought other genotypes perfectly integrated with the cultural environment and with positive effects on the qualitative and organoleptic characteristics (Schiavi *et al.,* 1991). Breeding activity is always depended on the availability of genetic variability, and thanks to the selection criteria applied by the farmers the biodiversity was saved (Schippmann *et al.,* 2002). The local populations are genotypes of high intrinsic value with a particular capacity of adaptability to their environment. These characters allow easier cultivation in Mediterranean basin compared to the varieties selected in different environments.Consumption of fruits and vegetables was associated with lower incidence and lower mortality rates of cancer in several human cohort and case-control studies for all common cancer sites (Steinmetz et Potter, 1996). Up to now, the association between intake of total fruits, vegetables, cardio, and cerebrovascular disease mortality is not definitively demonstrated, but under rigorously controlled

experimental conditions, fruit and vegetable consumption is associated with a decrease in blood pressure (Dauchet *et al*., 2009). The protection that fruits and vegetables provide against diseases such as cancer, cardio, and cerebrovascular illnesses, was attributed to various antioxidants contained in these fruits and vegetables, especially vitamins, including ascorbic acid and R-tocopherol. *Solanum melongena* L. fruits, commonly known as aubergine, eggplant, melenzana, garden egg, brinjal, and patlican, are very well known and consumed in various parts of the world and are ranked amongst the top ten vegetables in term of oxygen radical absorbance capacity due to their phenolic compounds (Cao *et al*., 1996).

The quantity and the quality of these compounds is significantly influenced by cultivar, environment, type of soil and growing conditions (Singh *et al*., 2009; Todaro *et al*., 2009). New cultivars of eggplant provide increasingly high production, but without taking into account the quality of the product. The local populations, although not comparable with the F1 hybrids with regard to production yields, can be used as niche products in areas suited to horticulture, especially for their adaptability to the characteristics of low energy inputs. Hence, the interest in development of ancient local populations that, if not adequately safeguarded and promoted, are likely to undergo a process of genetic erosion. Methyl bromide was used commonly as a soil fumigant. In order to protect the ozone layer from human pollution, the Montreal Protocol has regulated the use of this gas, as it has a high ozone depleting potential (ODP) (Ristanio and Thomas, 1997). The regular use of methyl bromide was banned in developed countries, and in 2015 will be banned in developing ones (Osteen, 2003). The prohibition of pest control with methyl bromide, and the lack of genetic material either tolerant or resistant to telluric biotic or abiotic stress, increases the interest in the practice of grafting vegetable species (Bletsos, 2005; Davis[a] *et al.*, 2008; Davis[b] *et al.*, 2008; King *et al.*, 2008; Miguel *et al.*, 2004). *Solanum torvum* Sw. is one of the recommended rootstocks for the eggplant that confers tollerance to a wide range of telluric pathogens (*Verticillium dahliae* Klebahn, *Ralstonia solanacearum* (Smith) Yabuuchi et al., *Fusarium oxysporum* (Schlechtend: Br) f. sp. *melongenae* Matuo and Ishigami, and *Meloidogyne* spp. root-knot nematodes, and *Ralstonia solanacearum*) (Bletsos *et al.*, 2003; Daunay *et al.*, 2008; Singh et Gopalakrishnan, 1997; King *et al.*, 2010). Thus, this is important to grow valuable genetic material by grafting onto

rootstocks that are either tolerant or resistant (Colla *et al.*, 2010; Sabatino *et al.*,2013). In this present work, four grafted and ungrafted eggplant ecotypes collected from Sicily were studied in order to characterize them by correlating agronomic, histological, histochemical results with HPLC-MS analysis.

5.2 Materials and methods

5.2.1 Plant material and cultivation

Four different eggplant ecotypes cultivated in Sicily in the provinces of Palermo, Trapani and Agrigento, (Figure 5.1; Table 5.1) were used as scion. *Solanum torvum* Sw. Australys selection (Agri Seeds s.r.l.) was used as a rootstock.

The study was carried out at the experimental fields of the Department of Agricultural and Forest Sciences of Palermo (Italy) and began on May 10th, 2011 with the transplantation of seedlings into the open field as a root ball. The soil on which it was performed the transplant was an Alfisol "Red Mediterranean Lands". The soil was prepared by making a medium-deep plowing (35 cm) and a reduction of the earth aggregates achieved by mechanical rotating means to prepare a good bed plant. It was carried out an improvement of the soil with manure mature at a dose of 40 t ha^{-1}. In addition, an irrigation system in micro scale with drip irrigation system placed under a black PE film thickness of 20 μm was designed.

The seedlings were transplanted with a distance of 0.50 m between plants along the row and 1.00 m in between the rows, with a density of 2 plants/m^2. A form of free breeding, trimming and flaking were adopted when become necessary. Moreover, were made of green pruning on grafted plants, capable of removing the shoots of rootstock. The amount of fertilizers units administered for fertigation was calculated on the basis of export performance (kg*t^{-1}), the expected production and allocation of soil mineral elements was 250 kg*ha^{-1} for nitrogen, 150 kg*ha^{-1} for phosphorous pentoxide and 250 kg*ha^{-1} for potassium oxide (La Malfa, 1990).

5.2.2 Sample preparation

Immediately after receipt of fresh eggplant samples, were washed thoroughly with cold tap water to remove adhering extraneous matter. After that, they were cut into small pieces using stainless steel knife. Eggplant pieces were spread on netted trays

and were dried in hot air oven at 60℃ till the equilibrium moisture levels were attained. Heating/drying oven with natural convection, Model Binder GmbH ED 115 Instruments, Tuttlingen. Dried eggplant pieces were mashed, passed through a standard 20 mesh size, and packed in PET jars until extracted and analyzed.

5.2.3 Sample extraction

To find the best extraction conditions and develop an inexpensive and efficient method. The extraction process was performed in batch mode. The extraction tests were carried out using different extraction times, temperatures, concentrations of the extraction solvents and solvent-to-solid ratio. Therefore, solvent-to-solid ratio was from 2:1 to 80:1, (v/w), extraction temperature from 10 to 60℃, extraction solvent concentration from 0.5 to 2%, extraction time from 0 to 1 hour. A comparative extraction was carried out using acidified alcoholic solvent (ethanol/water/HCl; 70:30:1, v/v/v) to determine total extractable polyphenols. Each extraction was transferred into a dark glass bottle with pumped nitrogen (a nitrogen sweep was used before and during filling), then stored at 4 ℃. The operation was repeated three times and all three extracts were combined and concentrated using Heildolph rotavapor instruments (D-91126 Schwabach, type: Heizbad WB eco, Germany) under reduced temperature and pressure. After concentrating, the extract was dissolved in 1 ml mobile phase (10% methanol and water), and filtered through a 0.45 μm PVDF syringe filter for HPLC and LC-MS analysis.

5.2.4 Instrument conditions

Phenolic compounds were identified using high performance liquid chromatography coupled with tendem mass spectrometry (HPLC/MS/MS). The HPLC system (LCMS-2020**,** Shimadzu, Milan, Italy), employed a Hypercil Gold C18 (3 μm particle size; 150 mm length 3.0 mm ID; Thermo Electron Co., Bellefonte, PA). Five microlitres of the extract was injected onto the column and the gradient elution was used for separations. Solvent A consisted of 10 % methanol in H_2O adjusted to pH 3.5 with formic acid. Solvent B consisted of 20 % H_2O (pH 3.5 with formic acid), 20 % methanol, and 60 % acetonitrile. At flow rate of 0.3 ml/min., the following linear gradient was used: 0 min, 100 % A; 10 min 20 % A; 20 min, 40 % A; 40 min, 0 % A;

held at 0 % A for 15 min. Five minutes of equilibration at 100 % A was allowed before and after each injection. Effluent from the column was introduced into a tendem mass spectrometer (triple-quadrupole. Micromass, Inc., Milan, Italy) equipped with pneumatically-assisted electrospray ionizations source (ESI).

Mass spectra were acquired in the negative ion mode under the following parameters: capillary voltage, 3 kV; source block temperature, 120 °C; desolvation gas temperature, 400 °C. Nitrogen was used as the drying and nebulizing gas at flow rates of approximately 50 and 450 l/h. For full-scan HPLC–ESI–MS analysis, spectra were scanned in the range of 50 to 1200 m/z. Data acquisi- tion and processing were performed using a Mass-Lynx NT 3.5 data system (Micromass Inc., Milan, Italy).

5.2.5 Calibration curves

Quantification of Polyphenols in eggplant peel by HPLC-PDA analyses was made against a calibration curve obtained with two reference solutions of Caffeic acid to quantify *N*-caffeoylputrescine, *N*-caffeoylputrescine derivatives, hydroxycinnamoyl amide, caffeoylquinic acid, 5-caffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid and 1-5-dicaffeoylquinic acid; and chlorogenic acid (Sigma) to quantify feruloylquinic acid, 3,5-caffeoylferuloylquinic acid, 4,5-caffeoylferuloylquinic acid, caffeoylsinapylquinic acid at the concentration from 0.05 to 250 µg/ml.

Correlation coefficient (R^2) of the standard curve of Caffeic Acid and of Chlorogenic Acid are reported in Table 2, were indicating a good linearity between the log transformed area and log-transformed concentrations within the tested concentration range.

Validation process of the RP-HPLC method was carried out following the EURACHEM guidelines.

The detection limit (y_D) and the quantitation limit (y_Q) were expressed for each analyte as signals based on the mean value (y_b) and the standard deviation (s_b) of the blank signal as follows:

$$y_D = y_b + 2t\, s_b \qquad y_Q = y_b + 10 s_b$$

where t is a constant of the t-Student distribution (one-tailed) dependent on the confidence level and degrees of freedom (df). A 95% confidence level was chosen.

For y_b and s_b determination, 9 blank measurements were performed by injection of 5 µL of blank. Limit of detection (LOD) and quantification (LOQ) values were obtained by projection of the corresponding signals y_D and y_Q through a calibration plot y = f(x) onto the concentration axis. Linearity of the method was established over 0.05-250 ppm by performing five HPLC replicates for each concentration level (ten equispaced concentration level).

5.2.6 Scanning light microscope (SLM)

The sample preparation for histochemical and histological studies was carried out using a combined methods (Jensen, 1962; Pearse, 1961; Ling-Lee et al., 1977; Wilson et Peterson, 1983). The thin sections from the epicarp and mesocarp portions of the eggplant were immediately fixed. They were embedded in paraffin and cut with a microtome "Minot" (Leitz). Then were stained with various histochemical tests: Sudan IV for lipids; FeCl3 for tannins and polyphenols; Lugol for starch, and Toluidine blue for anthocyanins and polyphenols. SLM was carried out using Light Microscope Leica DMLS 52, and the images were captured with digital ICCA camera connected to a computer.

5.2.7 Experimental design and statistical analysis

Treatments were defined by a factorial combination of the four scion ecotypes and two type of propagation techniques (grafted and ungrafted). A randomized block with three replicates of 10 plants per treatment was adopted like experimental design. A two-way fixed-effects general linear model analysis of variance (ANOVA) was used to determine the effects of different ecotypes and type of propagation techniques. In the following text, the main effects of each experimental factors will be separately reported, while any significant interaction which occurs between the factors will be shown in the tables. An α level of 95% was selected for the ANOVA. All percentage data prior to each processing were subjected to angular transformation (Φ = arcsin (P/100) 1/2). Data obtained were statistically analyzed using SPSS 14.0.

5.3 Results

A significance differences existed between total production, marketable production, and number of marketable fruits, depending on grafted and engrafted plant. By contrast, there was not significance differences in weight marketable fruits and waste production (table 5.3). Therefore, the highest total production/m^2 (7.73 Kg/m^2) was obtained by an higher number of marketable fruits (19.08). In terms of ecotype output, E1 was the most productive having provided productions significantly higher respect to the other three ecotypes (9.44 kg/m^2). E4 ecotype had the lowest level of production (5.00 kg/m^2) and with the highest waste production (18.18%). Excellent result was found in the ecotype E2 with a good level of production (7.90 kg/m^2) and with the lowest rejection rate (7.88%).

The number of marketable fruits/ m^2 differed significantly depending on the ecotypes, with the greatest number obtained from E4 (30.34). Whereas there was no statistically significant differences between ecotypes E1 and E2 and between E1 and E3. As regards, the average weight of fruits for the market, the ecotypes E1 and E3 have reached higher levels (676.00 g and 623.67 g) respectively, while the ecotype E4 showed the lowest values (165.50 g). This, may be is due to the berry shape typology.

The figure 2 shows typical HPLC chromatogram of the eggplant peel extracts. Demonstrated that the major phenolic acid (peak 6) was identified in ungrafted and grafted eggplants as 5-caffeoylquinic acid by comparison ultraviolet-visible and mass spectral data. Peaks 17, 8, and 7 were identified as 3-5-dicaffeoylquinic acid, 4-caffeoylquinic acid, caffeic acid conjugate, respectively. Peaks 12, 13, 14, 15, and 16 were not identified (table 5.4).

The results of HPLC analysis and quantification of 5-caffeoylquinic acid in 8 eggplant samples is presented in the table 2. The most abundant total polyphenol content were identified in the ungrafted sample E3 and the grafted sample E2 (65.85 , 47.29 µg/mL), respectively. The lowest total content was found in the ungrafted and grafted sample E1 (2.67, 5.86 µg/mL), respectively. The ecotype E3 presents the greatest amount of polyphenolic compounds and is the only ecotype in which the polyphenol content decreases in the aubergines from grafted plants. In the sample E3, 4,5 caffeoylferuloylquinic acid is also present in good percentage.

Scanning light microscope in the unstained peeled epidermis of ecotype samples E2, E3 and E4, showed several violet pigments. Sections of mature fruits showed an epidermis with the external tangential wall with cutin (Sudan IV test), cytoplasm and nuclei and several vacuolar incluses with polyphenols (Figures 5.3 and 5.4). Under the epidermis, we noted, a compact tissue without intercellular spaces with regular cells corresponding to hypodermis according to Dave (Dave et al., 1979) that is the stratified. Epidermis and vacuolar incluses were observed in samples E2, E3 and E4. Polyphenols scanning light microscope were absent in the sample E1 (Figure 5.5a), which was confirmed by their low level in the HPLC analysis. Under the ipoderm, in the mesocarp we observed a parenchyma with irregular cells and many intercellular spaces, that change their shape from rounded to elliptical, to elongated, and even to stellate, was accurately described in *S. mammosum* (Miller, 1969) These cells showed thin cell walls and vacuolized cytoplasm.

We also observed vascular bundles random, vacuolar incluses, starch, and seeds. The cells of pericarp didn't show cell wall modifications but in the various ecotypes the thickness of cell layers was different. In ecotype E4 ungrated and ungrafted we observed several hypodermic layers with pigments and cloroplasts. In the pericarp of E2, E3 and E4 samples, we observed, in fact, polyphenols and carbohydrates. Polyphenols presence in E2, E3 and E4 samples were observed on fresh material, on peeled epidermis and freehand sections.

5.4 Discussions

For all the production traits evaluated, the graft has proved a useful technique to increase the productive potential (Colla *et al.*, 2006), this being, from the physiological point of view in the eggplant, a propagation technique that has the purpose to allow the plants an increased absorption of water and nutrients (Lee, 1994). The significant differences found in the our ecotypes analyzed, concerning the average weight of the marketable fruits, may be is due to the berry shape typology.

5-caffeoylquinic acid by comparison ultraviolet-visible and mass spectral data was the major phenolic acid, which is confirmed by Whitaker & Stommel, 2003; Luthria & Mukhopadhyay, 2006; Singh *et al.*, 2009, and Luthria *et al.*, 2010.

The quantification of the three major Phenilamide compounds, N-Caffeoylputrescine, N-Caffeoylputrescine derivatives, Hydroxycinnamoyl amide, are related to improved growth and development in grafted plants (Alan *et al.*, 2007; Demirsoy, 2007; Sánchez-Rodríguez, 2011). These coincide with our results, by contrast, the ecotype E3 showed a lower phenylamide level with a higher total production. However, this result indicated that the rootstock did not substantially improve the phenolic metabolism, but the improvement of grafting is strictly related to scion-rootstock combination (Lee *et al.*, 2010).

Concerning scanning light microscope observations, the function of hypodermal cells would be provided mechanical support or, in some cases, participated in the dehiscence mechanism (Klemt, 1907; Dyki *et al.*, 1997). Our histochemical studies confirm those obtained by Tiwari (Tiwari *et al.*, 2009). The morphological and anatomical study performed on the fruits by Dave (Dave *et al.*, 1979) showed the origin from the ovary tissue characterizing the different types of cells forming the different tissues of the pericarp. Our observations, differ by them, only about the number of the layers of the epicarp and mesocarp. It is due, probably, to different genotype's features.

In Sicily (Southern Italy), were the vegetable cultivations still carried out mostly by traditional methods and modern cultivation techniques are adopted slowly, the grafting technique could help in the solution of many problems. Therefore, we consider the advantages of grafted plants, which offer significant increase yield and consequently higher profit, to be of value for farmers. This research showed that the use of *S. torvum* as rootstock, allowed an improvement as regard the cultivation performance as well as the qualitative characteristics. In fact, our results are important from the standpoint of improving on dry matter, marketable production, and number of fruits in the cultivation of eggplant by the used of grafting. This improvement is due to the augmentation of the marketable fruits number in the grafted ecotypes. Interaction between rootstock and genotype don't caused neither a reduction nor an increase in the fruits yield. The grafted eggplants displayed a behavior similar to that of the ungrafted genotypes, but it was the grafted itself that prompted the rise in total phenolic compounds with an exception in the sample of Marsala (E3), which caused a reduction of 58 %. This could be explained by the different ecotypes characteristics.

This result suggests that is essential to analyze multiple samples per ecotypes in order to draw any specific conclusion about the influence of grafting technique on phenolic content changes. In addition, the used of *Solanum torvum* as a rootstock increases the phenylamide content and improves the growth. Although, other processes could be involved, and more research are needed to clarify this point. The morphological analysis revealed important histological differences, such as the absence of polyphenols and the pigments in the epidermis of the sample E1. The micromorphological variations in peel eggplants failed to distinguish between the grafted and ungrafted samples. However, from the small sample examined, peel coat microstructure maybe useful in the identification and classification of the sectional and generic levels in the *Solanum melongena*.

Table 5.1 – Geographical origin and typology of the eggplant ecotypes used for the analysis.

Fruit shape	Ecotype	Region	District	Local name
Ovoid	E1	Sicily	Palermo	Bianca
Pyriform	E2	Sicily	Agrigento	Sciacca
Globular	E3	Sicily	Trapani	Marsala
Cylindrical	E4	Sicily	Trapani	Sicilia

Table 5.2 – HPLC-PDA-ESI-MS (negative ionization mode) polyphenolic fingerprint and values in μg/mL of major polyphenols in eggplants peel extracts (Bianca E1, Sciacca E2, Marsala E3 and Sicilia E4).

				Ungrafted				Grafted			
No.	**$[M-H]^-$**	**UV/Vis (nm)**	**PHENOLIC COMPOUND**	**E1**	**E2**	**E3**	**E4**	**E1**	**E2**	**E3**	**E4**
1	249	253, 291, 318	*N*-Caffeoylputrescine	0.27	0.86	1.94	1.84	0.72	2.23	0.79	1.74
2	249	288, 310, 321	*N*-Caffeoylputrescine derivatives[a]	0.25	1.78	2.03	1.31	0.64	3.91	0.61	1.56
3	472, (355)[#]	254, 291, 318	Hydroxycinnamoyl amide	0.28	1.45	1.58	1.42	0.59	9.55	0.39	1.32
4	353	224, 241, 329	Caffeoylquinic acid	0.72	12.85	22.07	4.75	3.55	19.66	4.47	8.72
5	353	250, 289, 314	5-Caffeoylquinic acid	-	0.15	2.89	0.36	< LOQ	1.25	0.05	0.95
6	367	247, 326	Feruloylquinic acid	0.06	0.55	3.49	0.30	0.13	2.12	0.81	1.43
7	515, (353) #	249, 323	3-5-Dicaffeoylquinic acid	-	0.78	2.44	0.33	-	2.18	0.02	0.59
8	515, (353) #	245, 327	4-5-Dicaffeoylquinic acid	< LOQ	< LOQ	1.65	0.34	-	0.87	0.02	0.62
9	515, (353) #	252, 325	1-5-Dicaffeoylquinic acid	< LOQ	-	6.24	0.01	< LOQ	0.85	0.01	0.66
10	529	255, 322	3-5-Caffeoylferuloylquinic acid	-	0.09	0.61	0.15	0.03	0.36	0.03	1.06
11	529	240, 324	4-5-Caffeoylferuloylquinic acid	0.02	1.69	18.96	0.16	-	1.38	0.18	0.84
12	559	244, 327	Caffeoylsinapylquinic acid	1.07	0.04	1.95	-	0.20	2.93	0.19	0.27
			TOTAL	**2.67**	**20.24**	**65.85**	**10.97**	**5.86**	**47.29**	**7.57**	**19.76**

[#]between parenthesis the daughter ions value for compounds 3, 4, 5, 7, 8, 9 are reported.
[a] Based on LC-MS mass fragmentation pattern and in conjuction with Singh (2009) and Whitaker (2003).

Table 5.3 – Yield production of four ecotypes of eggplant grafted and ungrafted.

		Average total production /m^2 [Kg]	Average marketable production/m^2 [Kg]	Average number of marketable fruits/m^2	Average Weight marketable fruit [g]	Waste production [%]
	Ungrafted	6.66 b	5.75 b	16,14 b	502,58 n.s.	14,25 n.s.
Type of plant	**Grafted**	7.73 a	6.62 a	19,08 a	487,50 n.s.	14,63 n.s.
	Bianca (E1)	9.44 a	8.00 a	14,12 bc	676,00 a	15,22 c
	Sciacca (E2)	7.90 b	7.27 a	15,54 b	515,00 b	7,88 d
	Marsala (E3)	6.44 c	5.38 b	10,42 c	623,67 a	16,48 b
Ecotype	**Sicilia (E4)**	5.00 d	4.09 c	30,34 a	165,50 c	18,18 a
Interaction		n.s.	n.s.	n.s.	n.s.	n.s.

In each column and for each fixed factor values followed by same letter are not statistically different according to Duncan test ($P \leq 0.05$).
For the interaction ns, *,**,*** indicate no significant or significant for P= 0.05 = 0.01 and = 0.001 respectively.

Table 5.4 – HPLC analysis of the major phenolic compounds extracted from eggplant peel.

N°	Phenolic Compounds	%
1	N-caffeoylputrescine derivatives	**5,54**
2	N-caffeoylputrescine	**3,22**
3	caffeoylquinic acid	**3,68**
4	dihydroxycinnamoyl amide	**2,34**
5	N,N'-dicaffeoylspermidine	**3,79**
6	5-caffeoylquinic acid	**52,57**
7	caffeic acid conjugate	**1,40**
8	4-caffeoylquinic acid	**7,51**
9	5-ciscaffeoylquinic acid	**5,88**
10	caffeic acid conjugate	**1,79**
11	3-acetyl-5-caffeoylquinic acid	**3,34**
12	4-5-dicaffeoylquinic acid	**ND**
13	3-acetyl-4-caffeoylquinic acid	**ND**
14	caffeic acid conjugate	**ND**
15	quercetin-3-β-glucoside	**ND**
16	quercetin-3-rhamnopyranoside	**ND**
17	3-5-dicaffeoylquinic acid	**8,63**
18	myricetin-3-β-galactoside	**0,30**

Figure 5.1 – Ecotype Bianca E1, Sciacca E2, Marsala E3 and Sicilia E4

Figure 5.2 – HPLC chromatrogram (325 nm) extracted from eggplant peel reveling the presence of 18 peaks identified based on the ultraviolet and mass-spectral data: (1) N-caffeoylputrescine derivatives; (2) N-caffeoylputrescine; (3) caffeoylquinic acid; (4) dihydroxycinnamoyl amide; (5) N,N'-dicaffeoylspermidine; (6) 5-caffeoylquinic acid; (7) caffeic acid conjugate; (8) 4-caffeoylquinic acid; (9) 5-ciscaffeoylquinic acid; (10) caffeic acid conjugate; (11) 3-acetyl-5-caffeoylquinic acid; (12) 4-5-dicaffeoylquinic acid; (13) 3-acetyl-4-caffeoylquinic acid; (14) caffeic acid conjugate; (15) quercetin-3-β-glucoside; (16) quercetin-3-rhamnopyranoside; (17) 3-5-dicaffeoylquinic acid; (18) myricetin-3-β-galactoside.

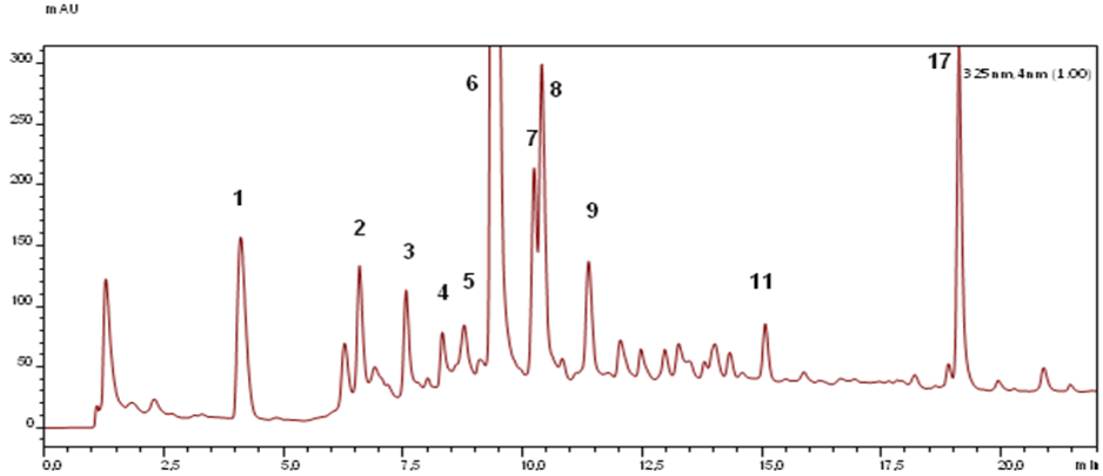
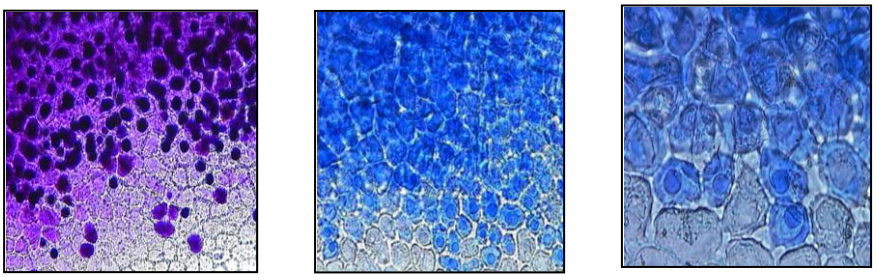
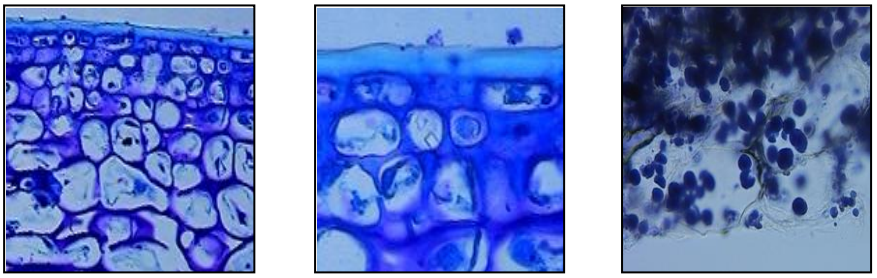


Figure 5.3 – Ecotype Sicilia ungrafted. a) Peeled epidermis (40X) unstained. Pigments in cells, b) Peeled epidermis (40X) Presence of polyphenols in cells. Test: FeCl3. and c) Peeled epidermis Presence of polyphenols in vacuoles(100X). Test: FeCl3



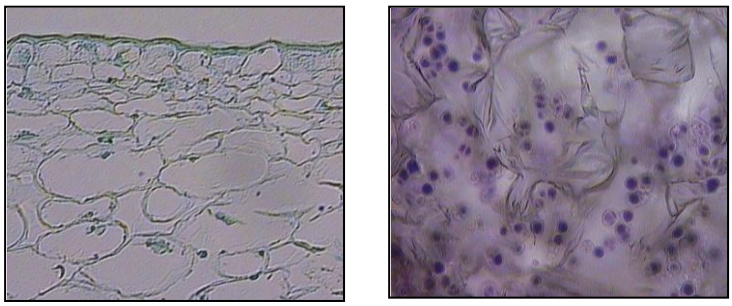
A **B** **C**

Figure 5.4 – Ecotype Marsala ungrafted. a) Cross section (40X) of pericarp. Presence of polyphenols in cells. Test: Toluidine blue, b) Cross section (100X) of pericarp. Presence of polyphenols in cells. Test: Toluidine blue and c) Hand section (40X). Presence of starch Test: Lugol.



A **B** **C**

Figure 5.5 – Ecotype Bianca ungrafted. a) Cross section (40X) of pericarp. Absence of polyphenols in cells. Test: Toluidine blue and b) Ecotype Sicilia ungrafted. Hand section (40X). Presence of starch Test: Lugol.



A **B**

6

Use of Unrooted Grafted Cuttings for Watermelon: Effect of Healing Duration and Transportation Temperature on the Cutting and Finished Plant Quality

6.1 Introduction

Vegetable grafting can be described as the joining of two compatible plants together to form one; using the shoot of one plant and the root system of another. Vegetable grafting can provide the benefit of increased environmental stress tolerance and yield in addition to resistance to soil-borne disease and pests (Lee and Oda, 2003; Davis *et al.* 2008; Schwarz *et al.*, 2010). Among various vegetable crops that have been grafted worldwide, watermelon (*Citrullus lanatus*) is the crop species for which grafting is used at the highest degree. For example, in Japan, Korea and southern Spain, more than 90% of watermelon crop production now utilizes grafting (Lee *et al*., 2010), mainly for controlling Fusarium disease associated with continuous production without crop rotation. Commonly used rootstocks for watermelon are bottle gourd (*Lagenaria siceraria*) and interspecific squash (*Cucurbita maxima x Cucurbita moschata*). Interspecific squash rootstocks are known to be effective against *Fusarium* (Miguel *et al*., 2004), and sudden wilt/vine decline (Edelstein *et al*., 1999) for cucurbits. They are also vigorous and exhibit tolerance to environmental stresses such as cold temperature (Justus and Kubota, 2010; Okimura *et al*., 1986). Therefore, use of interspecific rootstock is often preferred, although use of bottle gourd may be more common in some countries in order to avoid possible alteration in fruit quality (T. Itagi personal communication; Rouphael *et al*., 2010).

Despite the benefits, especially in North America, use of grafting is limited due to the undeveloped nurseries' capacity of this labor intensive production and the high plant production costs. The necessity of the large skilled labor input associating with the potentially large demand to serve industrial open-field production is another reason why grafting is not commonly utilized (Kubota *et al*., 2008). Possible solutions may include the use of grafting robots that mechanically graft seedlings at high rates. However, grafting robots require uniform seedlings stock, high input cost, and are not widely available in the US (Kubota *et al*., 2008). Especially during the time when the local demand of grafted plants is still small, it is challenging for nurseries to establish grafting capacity that requires large investment and modifications of production systems. Nurseries could distribute their transplants to producers in remote locations, but transportation costs and distance that allows them to deliver plants without causing negative impact to the plants is limited. Grafted tomato seedlings are transported for such a long distance using refrigerated trucks (Kubota and Kroggel, 2006) from Canadian nurseries to US greenhouse producers (who can afford the additional costs). Potential solution for such a situation is the separation of primary nurseries for the critical grafting processes and secondary nurseries for finishing the plug transplants. In this scenario, primary nurseries can harvest grafted cuttings and ship them to secondary nurseries in remote locations to re-root and finish them to distribute to their local producers. A similar business model is widely practiced in vegetatively propagated species of floriculture and ornamental crops in North America and Central Europe (Papaka *et al*., 2007). For example, unrooted cuttings of pelargonium used for transplant production in Central Europe are transported from various countries including Southern Europe, North Africa, and Central America (Druege *et al*., 2004).

For vegetable crops, Shiraki *et al*. (1999) proposed the use of unrooted grafted cuttings for tomato and cucumber in Japan and showed that the cuttings could be shipped immediately during warm season while they required a minimum of 24 hours to heal before shipping for the propagation in cold season. The authors are aware of a limited number of commercial nurseries practicing the use of grafted cuttings for vegetable crops in East Asia (e.g., Shibuya *et al*., 2008). But unfortunately, feasibility of this technique in North American fruiting vegetable production has not been

examined yet. In Japan, grafted cuttings are packed for shipping immediately after the grafting (without healing them). The prerequisites of this practice are: 1) the fragile cuttings can survive through the long distance transportation without any negative impact, and 2) recipients of the cuttings (secondary propagators) have the facilities for healing the grafted cuttings. However most existing US nurseries do not have sophisticated controlled environment systems for healing the grafts. Use of grafted cuttings can allow nurseries to use expensive freight system to reach the long distance in one to three days. However, use of third parties' services may increase the risk of damaging freshly grafted cuttings unless they are packed in a protective manner. Therefore, we believe that grafted cuttings be completed with the first critical stage of healing process to reduce the potential loss. Exact number of days that cuttings need to be healed is crucial information to adopt this technique.

Other important information is the packing methods and conditions for long distance transportation of any planting materials, since transportation conditions affect the plant quality and growth. Much research has been done for evaluating shipping conditions for ornamental species (Conover, 1976; Wang, 1987; Lopez and Runkle, 2008; Rapaka *et al*., 2008). A typical issue associated with unfavorable shipping conditions of unrooted cuttings is rapid leaf senescence and impaired root formation (Rapaka *et al*., 2008; Serek *et al*., 1998).

In the present study, we examined grafted watermelon cuttings ('Tri-X 313' scion and 'Strong Tosa' interspecific hybrid squash rootstock') under 'simulated transportation temperature' in a growth chamber to find their healing requirement and transportation temperature.

6.2 Materials and Methods

6.2.1 Plant materials and growing conditions

A 7.30 x 14.60-m, north-south oriented, A-frame compartment located within a acrylic-glazed gutter connected greenhouse structure at The University of Arizona (Tucson, AZ, USA) was used for the experiment. The greenhouse was equipped with a fan-and-pad evaporative cooling, high-pressure fogging and over-head air heating systems.

The ‘Tri-X-313’ watermelon and ‘Strong Tosa’ interspecific squash (Syngenta/Roger Seeds, Boise, ID) were seeded into 128-cell trays containing commercial substrate mix (Fafard #2, Agawam, MA, USA). After germination, the seedlings were moved on the bench inside a greenhouse, and the target air temperature was set at 25/18 °C (day/night). ‘Strong Tosa’ seeds were sown 2 day after seeding ‘Tri-X-313’ watermelon, so that both would reach the growth stage suitable for grafting at the same time. Once the plants expanded their cotyledons they were irrigated with a half-strength modified Hoagland's nutrient solution (EC 1.2 dS m^{-1} and pH 6.0) as needed. The basal components of the nutrient solution were 95 N, 23.5 P, 175 K, 100 Ca, 30 Mg, 44.5 Cl, 58 S, 1 Fe, 0.3 Mn, 0.15 Zn, 0.2 B, 0.05 Cu and 0.05 Mo in mg/l (ppm). Grafting was performed using a single cotyledon grafting method (a splice grafting method leaving one cotyledon for rootstocks, as described in Lee and Oda, 2003) using a spring loaded grafting clip (Product ID 9032, Johnny’s Seeds, Waterville, Maine 04903) to hold the scion and rootstock together at the graft union. For healing, the grafted seedlings were immediately enclosed inside a transparent plastic box and moved into a chamber (Model 2015; VWR International, Cornelius, OR) maintaining darkness at a temperature of 29°C and 95 – 100% RH. After 24 hours the level of the light was increased to at 55 µmol m^{-2} s^{-1} photosynthetic photon flux (PPF). Light source was T5 white fluorescent tubes mounted horizontally above the storage box. On the beginning of 5^{th} day in healing, the lid of plastic box was opened slightly for acclimatizing the seedlings under lower humidity (80-90%). During the healing, a self-logging sensor (temperature and relative humidity) was placed inside the box to monitor air temperature and relative humidity inside the box.

6.2.2 Harvesting procedure and simulated transportation conditions

Grafted cuttings were harvested by excising at 6 cm below the grafted union so that uniform cuttings will be obtained with sufficient length of hypocotyl for re-rooting. Harvesting time was 0 (immediately after grafting), 1 (end of the dark period), 3, 4, 5 (end of the high humidity), or 7 days in healing. The harvested cuttings were gently covered with a moist paper towel and inserted into a closed plastic bag for maintaining moisture. The bags each with 5 grafted cuttings were placed in vertical orientation with apical meristem down to minimize gravitational bending inside dark

chambers controlling air temperature at 12 or 20°C for 72 hours. The duration of 72 hours was selected based on the likely duration for the time necessary for the air freight and associating handlings of cuttings in North America. The temperature inside each storage chamber was monitored using T-type thermocouples (Gauge 24 or 2.1 mm in diameter) connected to a data logger (Model CR 10X, Campbell Scientific. Logan, UT, USA).

In addition to the treated cuttings, two control treatments were prepared as reference. First control group represents standard grafted seedlings that were produced in a conventional method (without cutting, without the 72 hour temperature treatment), and the second control group represents the unrooted grafted cuttings that were harvested after completing the 7-day healing and immediately placed in rooting conditions without applying the 72 hour treatment (the non-treated control). Timeline of the six treatments and two control groups are shown in Fig. 1.

6.2.3 Rooting conditions

After 72 hours of the simulated transportation treatment, all un-rooted grafted cuttings were transferred into the greenhouse for rooting and final hardening as plug transplants. No plant growth regulators were applied. Cuttings were inserted into the fresh moist substrate (Sun Gro Sunshine Professional Mix 3, Bellevue, WA) in the 98-cell trays. Plants were placed into clear boxes, with a layer of water for humidity, (rooting trays elevated above water) and placed under 85% shade to prevent the closed boxes from getting too hot. On the 6^{th} day, the covers were loosened to start acclimatizing the cuttings. Covers were removed on the 7^{th} day. Plants were moved to the bench with 50% shade for 2 days and then placed under the full sun to finish for additional 3 days. The rooted grafted cuttings were then transplanted into 4-L plastic pots filling the commercial substrate mix (Sunshine Mix #1, Sun Gro Horticulture, Agawam, MA) until first female and male flowers were open.

6.2.4 Measurements

The first set of observations was taken after completion of the 72 hour temperature treatments, for the number of true leaves (with leaf blade length greater than 1 cm) and overall visual quality. The second observation was performed after completing

the 7-day rooting process, and the data of the scion epicotyl length, the number of true leaves (with leaf blade length greater than 1 cm) and the rooting percentage. The third observation was performed after the 5 days in the plug finishing stage with destructive analysis for the scion epicotyl length, number of leaves, and fresh and dry weight of the aerial part of seedlings. Dry weight was measured for the samples dried inside an oven set at 80°C for 3 days. The last set of observations was for number of days to flower after transplanting into the larger pots.

6.2.5 Experimental design and statistical analysis

The experiment was conducted during Sept 6th – Nov 10th, 2012 as the 3rd trial after the preceding two preliminary experiments using the same experimental materials but different timings to obtain measurement variables. Overall, the trend in plant response to the treatments was similar but we could not pool the data due to the difference in the experimental timelines. Data was analyzed by JMP software (version 7.0, SAS Institute, Cary, NC, USA). Each bag of 5 cuttings placed inside the temperature controlling chamber was considered as a replication (n=4). For fresh and dry weight, 4 plants were used per replication, and 1 plant per replication was subject to grow-on to flower.

6.3 Results and Discussion

6.3.1 Greenhouse environmental conditions

The environmental conditions inside the greenhouse during the seedling growth period before grafting had an average day/night air temperature of 25.3±2.1 (s.d.) /21.6±2.1 (s.d.) °C with an average daily photosynthetic light integral (DLI) of 21.2±4.5 (s.d.) mol m^{-2} d^{-1}. The healing conditions recorded were 29.8±0.9 (s.d.) °C air temperature and 99.9±0.2 (s.d.) % relative humidity. The day/night air temperature inside the rooting box during the rooting was 25.6±2.7 (s.d.) / 21.2±1.8 (s.d.) °C. The environmental conditions inside the greenhouse during the post-rooting growth period had an average day/night air temperature of 23.2±1.3 (s.d.) /17.9±1.8 (s.d.) °C with an average daily photosynthetic light integral (DLI) of 20.5±0.9 (s.d.) mol m^{-2} d^{-1}.

6.3.2 Grafted cutting visual qualities

Grafted cuttings harvested immediately after grafting (Day 0 in healing) were most fragile and much care was needed for handling them. Therefore we confirmed that these fragile cuttings were not suitable for shipping for long distance. We also noticed that spring type grafting clips could be problematic as they can damage other cuttings by mechanical compaction/frictions. After 4 days in healing (Day 4), most grafted seedlings started expanding the first true leaf, an early sign of successful grafting. As a consequence, grafted cuttings after 4 or more days in healing were easier to handle than those after less amount of time in healing.

The temperature of 72 hour treatment to simulate the transportation affected the visual quality of grafted cuttings. There was higher percent bending of scion hypocotyls observed under 20°C than under 12°C (Fig. 6.2, data not shown). There was no other particular difference in visual quality observed for grafted cuttings after the 72 hour temperature treatment, as affected by duration of healing at either temperature.

6.3.3 Rooting and regrowth of grafted cuttings

All cuttings developed roots at the end of the hardening stage, regardless of duration in healing or temperature treatment (Table 6.1, Figure 6.3). Post-rooting growth was significantly affected by duration in healing (Table 6.1) but not by temperature treatment. There were no interactions between duration in healing and temperature. Shoot fresh (data not shown) and shoot dry weight of the cuttings were not significantly different from those of untreated control, when harvested after 4 or more days in healing. Dry weight tended to be less when cuttings were harvested after longer time in healing (except those after one day in healing). The reduction in dry weight was probably associated with the healing conditions, which could be further optimized to achieve at least null or positive carbon balance to the plants and thereby no loss in dry mass during the healing. In various studies on storing plants, maintaining dry mass has been recognized as important when plants were placed under conditions to suppress growth for prolonged period of time, such as in low temperature storage (e.g., Kubota *et al.*, 1995). Under the continuous lighting conditions, the minimum light intensity to maintain the null carbon balance is the

light compensation point at the temperature used in the system. For healing, information is limited with regard to the optimum light intensity. Johnson and Miles (2011) demonstrated that healing chamber designs affect the conditions and thereby the grafting success. They also found that watermelon seedlings were more sensitive to humidity control than tomato or eggplant seedlings.

Scion epicotyl length and number of true leaves were smaller when grafted cuttings were harvested after 4 days or less in healing (Table 6.1), suggesting that the scion growth and development were suppressed when the grafted cuttings were harvested during the time grafts were not taken.

6.3.4 Finished plug transplant qualities

During the rooting stage, watermelon cotyledonary leaves became yellow and died off, which was more pronouced when the grafted cuttings treated at 20°C than at 12°C (data not shown). This is likely attributed to the higher respiratory loss of carbohydrate reserved in cotyledons as well as potentially greater ethylene accumulation in cuttings during the 72-h temperature treatment at the higher temperature of 20°C. Rapaka *et al.* (2008) investigated the role of ehytlene action in postharvest leaf senescence of pelargonium cuttings and showed that ethylene sensitivity increased with decreasing pre-harvest endogenous carbohydrate status of the cuttings.

The gravitational bending of scion hypocotyls during 20°C temperature treatment made the overall quality of finished plug transplants less favorable than those treated at 12°C. Healing time before havesting the grafted cuttings seems to affect the finished plug transplant qualities also. The degree of senescence of scion's cotyledonary leaves seemed to be pronounced when the grafted cuttings were harvested after longer period of healing time (data not shown). There was no such leaf senescence observed for the grafted seedlings produced conventionally without using cuttings (Conventional control, Figure 6.3). Together with the evidence of high temperauture caused leaf senescence stated earlier, this may indicate that there is a threshold length in total length of healing and the rooting at a given temperature. The difference in plant quality betweeen 12°C and 20°C and between different timings of harvesting cuttings did not become visible upon removal from the chamber.

Therefore, improving rooting conditions may effectively prevent this leaf senescence. Use of misting or fogging combined with shading in a greenhouse or a controlled environment room may be more suitable for rooting these grafted cuttings, instead of contained plastic enclosure used in our experiment.

It was of interest that 12℃ is generally considered as a chilling temperature for watermelon, but scions survived and maintained the regrowth capacity for the 72 hours exposure to the low temperautre. Rather, the low temperature kept the grafted cutting quality higher by preventing gravitational bending as well as browing/yellowing the cotyledonary leaves. It is possible that the interspecific squash used as rootstock helped maintaining the quality of scion tissue, but this aspect needs to be further investigated by testing various combinations of scion and rootstock genotypes under varied temperature conditions. A separate study at the Univeristy of Arizona also indicated that watermelon seedlings could be stored at 12°C without causing significant reduction in quality and regrowth ability for a short period of time (one to four weeks, depending on the growting conditions) (Spalholz, 2013).

6.3.5 Days to first male and female flowers

Neither treatment temperature nor the harvest timing affected the days to first male and female flowers anthesis after transplanting (Table 6.1). However, there were significantly less number of leaves developed under the first femal flower when the cuttings were harvested after 1, 4, and 7 days in healing ($P \leq 0.05$) (data now shown). The establishment of the grafted plants and the effect of propagation methods on the performance after the transplanting need to be investigated further in order to develop the propagation and distribution using grafted cuttings.

6.3.6 Growth comparison between rooted grafted cuttings and conventional grafted seedlings.

All the growth and developmental variables (except number of leaves and plant height) measured for the rooted grafted cuttings (non-treated control) were comparable to those for the conventionally grown grafted seedlings (Table 6.2, Figure 6.3). The advanced leaf development observed for the non-treated control plants could be attributed to possible enhancement of cytokinin synthesis associated with the

formation of advantitious roots. Sallaku et al. (2012) compared grafted watermelon growth grafted in two different ways (with and without root removal) and demonstrated that grafting without roots to form advantitious roots promoted post-grating growth and stand establishment. A cytokinin assey conducted by Sallaku et al. suggested a higher level of cytokinins produced by the formation of many adventitious roots. Howver, in our experiment, 7 days in healing followed by the 7 days in rooting after cutting lowered the visual quality of finished plugs due to the yellowing of scion cotyledons, while healing and rooting occurred .

6.4 Conclusions

Rooting ability of grafted cuttings was not affected by duration of healing nor 'transportation' temperature. Post rooting growth was, however, suppressed by shorter time in healing, despite that significant reduction of fresh and dry mass was observed after longer time in healing. Temperature did not affect rooting or regrowth capacity but notably affected the quality of grafted cuttings as well as the finished transplants (rooted grafted cuttings). Based on our finding, we recommend that grafted cuttings be harvested after 5 or 7 days and ship in a box preventing temperature increase during transportation. Further studies are under way to conduct the shipping trials of grafted watermelon cuttings between Arizona and northern regions.

Table 6.1 – Effects of harvesting timing of grafted watermelon cuttings on the percent rooting, shoot dry weight (DW), scion epicotyl length and number of true leaves developed on the grafted cuttings at the end of 5-day plug finishing stage subsequent to the 7-day rooting stage and the days to female and male flower anthesis after transplanting. Untreated control cuttings were those that did not experience the 72-hour simulated temperature treatment in the growth chamber.

Days in healing to harvest	Percent rooting in GH	DW (g)	Epicotyl (mm)	# Leaves	Days to 1st female flower after transplanting	Days to 1st male flower after transplanting
0	100^{NS}	0.23^{**}	10.7^{**}	2.2^{**}	29.3^{NS}	29.0^{NS}
1	100^{NS}	0.21^{NS}	7.0^{**}	2.1^{**}	28.1^{NS}	30.3^{NS}
3	100^{NS}	0.23^{*}	12.0^{**}	2.9^{**}	29.3^{NS}	30.4^{NS}
4	100^{NS}	0.19^{NS}	12.7^{**}	2.8^{**}	29.3^{NS}	31.4^{NS}
5	100^{NS}	0.18^{NS}	17.2^{NS}	3.3^{NS}	28.8^{NS}	31.1^{NS}
7	100^{NS}	0.18^{NS}	24.4^{NS}	3.7^{NS}	28.1^{NS}	31.3^{NS}
7 (Untreated control)	100±0	0.20±0.01	22.2±3.0	3.6±0.2	31.0±1.7	32.0±0.4

^{*,**,NS} Means significantly or non-significantly different at $p<0.05$ or 0.01 from the corresponding value of untreated control by t-test, respectively.

Table 6.2 – Growth and developmental parameters of grafted watermelon transplants produced by using un-rooted grafted cuttings without the 72-h temperature treatment (the non-treated control) and a conventional method for grafted seedlings (the conventional control) without using un-rooted grafted cuttings.

	At planting		After planning			
	Epicotyl length (mm)	Number of leaves	Days to 1st female flower after transplanting	Days to 1st male flower after transplanting	# Leaves under the 1st female flower	# Leaves under the 1st male flower
Rooted grafted cuttings (non-treated control)	22.2±3.0	3.6±0.17	31.0±1.7	32.0±0.4	9.8±0.6	7.8±0.9
Grafted seedlings (Conventional control)	17.2±1.7	3.0±0.08	32.8±1.5	32.8±0.6	10.8±0.9	8.0±0.4
T-test (P value)	*0.192*	*0.027*	*0.466*	*0.356*	*0.382*	*0.816*

Figure 6.1 – Experimental treatments and timelines. Conventional seedling production consisted of 2 days in germination (marked as 'G' in the diagram) and 5 and 7 days in greenhouse for 'Strong Tosa' rootstock and 'Tri-X-313' scion, respectively ('S/R'), followed by grafting, 7 days in healing ('H'), and 5 days in hardening/finishing ('Hard') the grafted plug seedlings. Grafted cuttings were harvested in different timings (0, 1, 3, 4, 5, and 7 days in healing, DIH) and treated under 72 hour in darkness at 12°C or 20°C for simulated shipping temperature ('72'), after which they were under 7 days in rooting condition ('Root') before the 5 days in hardening/finishing the transplants. The non-treated control was the grafted cuttings harvested and immediately rooted without experiencing the 72 hour treatment.

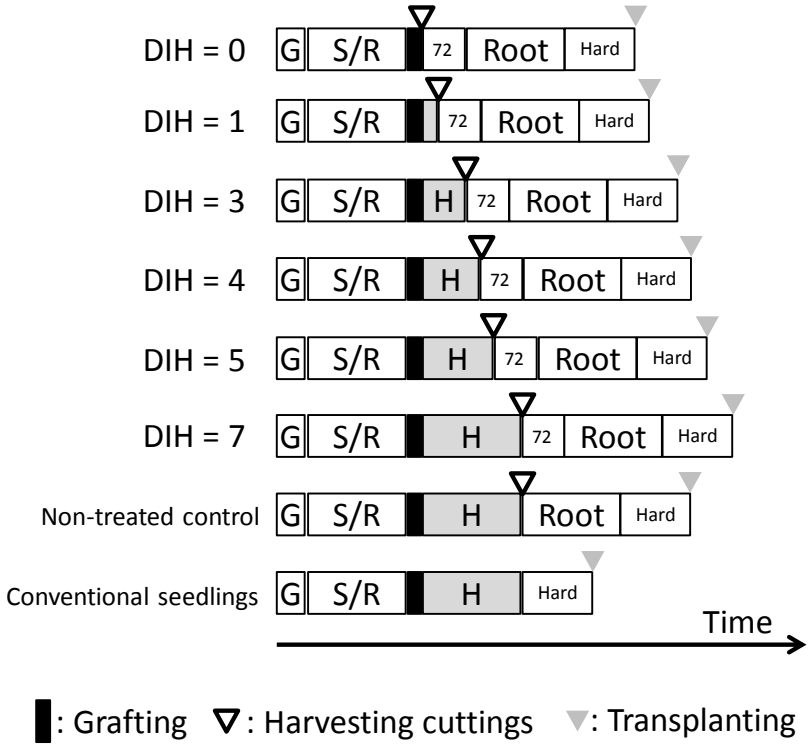


Figure 6.2 – Grafted cuttings after 72 days in darkness at 12°C (a) and 20°C (b) air temperature. Cuttings were harvested after 5 days in healing and packed inside plastic bags with moist paper towel. More cuttings were bent when treated at the higher temperature of 20°C.

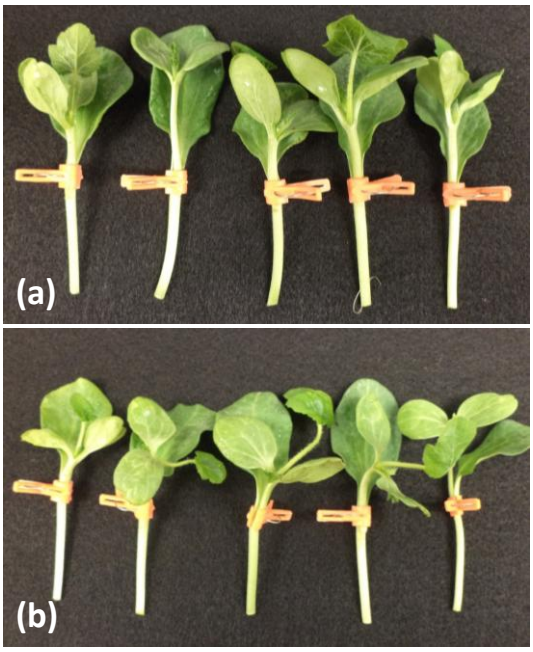


Figure 6.3 – Grafted watermelon transplants produced by using (a) un-rooted grafted cuttings without the 72-h temperature treatment (the non-treated control) and (b) conventional methods for grafted seedlings (the conventional seedlings) without using un-rooted grafted cuttings.

7

Production of Unrooted Grafted Cuttings of Eggplant and Field Evaluation

7.1 Introduction

The high demand for off-season vegetables and the intensive cropping systems with limited crop rotations, has determined a buildup of detrimental factors (biotic or abiotic) that can substantially reduce yield and crop quality. The abiotic factors include environments that are too cold, wet or dry, hypoxia, salinity, heavy metal contaminations, excessive and insufficient nutrient availability, and soil pH stress (Abdelmageed and Gruda, 2009; Ahn *et al.*, 1999; Estañ *et al.*, 2005; Venema *et al.*, 2008; Rivero *et al.*, 2003a,b; Savvas *et al.*, 2010). These unfavorable conditions may cause attacks from pests and diseases. For the past, methyl bromide (MB) was the fumigant of choice for many replant soil applications. The reasons primarily focus on the broad spectrum of activity of the fumigant, its high vapor pressure facilitating distribution through the soil profile, cost-effectiveness, and comparatively short plant-back intervals. Historically, MB was primarily used to control lethal soil-borne pathogens such as *Verticillium dahliae* (Wilhelm and Paulus AD, 1980), but it also can provide excellent control of nematodes and a broad spectrum of weeds. With the Montreal Protocol, that was established to regulate the use of gases with high ozone depletion potential (ODP) in order to protect the ozone layer from human pollution (Ristanio and Thomas, 1997; UNEP, 2006), the grafting technique, has increasingly represented an environmentally friendly viable alternative to solve the vegetable plant problems related to the biotic and/or abiotic stress (Lee and Oda, 2003; Davis *et al.* 2008; Schwarz *et al.* 2010; Savvas *et al.*, 2010). During recent years, the use of

grafted vegetable seedlings has gained interest in many countries (Lee and Oda, 2003). In fact, the cultivated area of grafted *Solanaceae*, including a number of important annual fruit-crop plants such as tomato, eggplant and pepper, has increased. Eggplant (*Solanum melongena* L.) is one of the most widely cultivated crops in tropical and temperate regions around the world (Bletsos *et al.,* 2003). *Solanum torvum* Sw. is considered one of the most suitable rootstocks for eggplant, providing resistance to a large number of soil pathogens and nematodes (*Verticillium dahliae*, *Ralstonia solanacearum*, *Fusarium oxysporum* f. sp. *Melongenae* and *Meloidogyne* spp. root knot nematodes) (Bletsos *et al.*, 2003; Daunay, 2008; Singh and Gopalakrishnan, 1997; King *et al.,* 2010). However, the nursery production of eggplants grafted onto *S. torvum* has some issues due to the low germination of rootstock seeds (Gousset *et al*., 2005) and the slow growth and development of seedlings. These critical points reduce the efficiency of nursery production. Unrooted cuttings harvested from stock plants could be an alternative propagation material to obtain *S. torvum* rootstocks that might allow to overcome nursery production issues. The purpose of this work was to evaluate eggplant suitability to be propagated by unrooted grafted cuttings using *S. torvum* as rootstock and the influence of this propagation technique on plant yield and fruit quality.

7.2 Materials and methods

The suitability of eggplant to be propagated by unrooted grafted cuttings, using *Solanum torvum* as rootstock, was assessed with nursery and field trials performed in 2012 and 2013 in the experimental farm of the Department of Agricultural and Forest Sciences - University of Palermo - Italy (38° 9’ 23’’ N, 13° 20’ 2’’ E; altitude 48 m). The eggplant cultivar Birgah was used as the scion variety as well as the ungrafted control (seedling control and unrooted cutting control). Two propagation techniques of the rootstock were evaluated using *S. torvum* and ‘Birgah’ eggplant (self-grafted): unrooted cuttings or seedlings (table 7.1).

7.2.1 Plant materials and nursery production

The nursery trials were conducted into a controlled-temperature greenhouse equipped with mobile benches, high pressure fogging and overhead air heating systems.

The rootstocks of *S. torvum* and eggplant (self-grafting) consisted of seedlings or unrooted cuttings.

Seeds of 'Birgah' eggplant and *S. torvum* were sown into polystyrene trays with 40 compartments filled with a commercial substrate (Technic nr. 3 Dueemme marketing s.r.l., Reggio Emilia, Italy, Europe). After sowing, trays were placed on mobile benches at a temperature ranging from 24°C to 26°C. Nursery trays were manually watered every day in order to maintain the substrate at field capacity. After germination air temperature was set at 25/18°C (day/night). 'Birgah' seeds were sown 25 days after *S. torvum* seeds, so that both would reach the growth stage suitable for grafting at the same time. After the plants showed cotyledons they were fertigated with a nutrient solution (EC 3.00 mS cm^{-1} and pH 6). The basal components of the nutrient solution were 20 NO^{3-}, 0.90 $H_2PO_4^-$, 3 $SO4^{2-}$, 6.30 K^+, 6.30 Ca^{2+}, 4.30 Mg^{2+}, 15 Fe^{3+}, 7 Mn^{2+}, 60 B^{3+}, 7 Zn^{2+}, and 0.7 Cu^{2+} in mM l^{-1}.

To produce unrooted cuttings of *S. torvum*, a stock plants field was realized on 2011. *S. torvum* plants were fertigated and pruned repeatedly with topping cuts to increase the number of shoots suitable for grafting. Cuttings of about 15 cm of length and a diameter of 2 mm were harvested when eggplant scions were ready for grafting. Eggplant unrooted cuttings were produced by cutting 50 days old seedlings at the root collar.

Grafting was performed by tube grafting method as described by Lee *et al.* (2010), but using grafting clips rather than silicon tubes. The grafted seedlings were immediately misted and placed inside a humidity chamber realized with a small plastic tunnel inside the greenhouse to permit healing. During the first 24 hours the grafted plants inside the humidity chamber were kept in the dark at 29°C and 95-100% RH. Afterwards, the light level was increased to 55 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) using T5 white fluorescent tubes installed horizontally above the humidity chamber. After 4 day from grafting, plants were acclimatized to the natural conditions of the greenhouse by slowly dropping the humidity (RH 70-80%) during 3 days, until they were ready for transplant. Immediately after grafting, the unrooted grafted cuttings were plugged in a 40– cell trays containing commercial substrate mix (Thechinic Dueemme marketing s.r.l., Reggio Emilia, Italy, Europe) and placed into the greenhouse under mist propagation conditions in order to allow healing and

rooting (27/25°C, substrate/air; about 100% RH). After roots emission, plants were acclimatized to the natural conditions of the greenhouse by slowly dropping the humidity (RH 70-80%) until they were ready for transplant.

Forty plantlets or unrooted cuttings of each trial were grafted.

In order to evaluate quality of grafted plants, the number of leaves longer than 1 cm and the height of plants were recorded at the grafting day, at the end of the healing and rooting and after 5 days from when plants were ready for transplant. During the 2^{th} set of observations, the grafting success, and the rooting percentage were also recorded. In addition, on 5 plants for each trial, fresh and dry weight of the above-ground part and length, fresh and dry weight of the roots were calculated. The dry matter was calculated by drying samples in a thermo-ventilated oven at 80°C for 3 days until constant weight was reached.

The timeline of the nursery propagation was also recorded.

Treatments were defined by a completely randomized design with three replicates per treatment, each consisting of 40 plants Statistical analyses were performed using a one-way ANOVA and the means were compared by Duncan multiple-range test.

7.2.2 Field trials

Eggplant plants non-grafted, self-grafted onto seedlings or unrooted cuttings and grafted onto seedlings or unrooted cuttings of *S. torvum* were transplanted for the field trials on 15 May 2012 and 2013 to a soil (alfisols "Red Mediterraenan soils") mulched with a 20μm black PE film. Plants were spaced 1 m between rows and 0.5 m apart within the row (2 plants m^{-2}) and drip irrigated. All cultural practices recommended for eggplant cultivation were uniformly adopted according to crop requirements. Fertilization was applied with drip irrigation throughout the growing cycle; the amount of nutrients, calculated on the basis of theoretical uptake, expected yields and mineral elements in soil, was: N 250, P_2O_5 150 and K_2O 250 kg ha^{-1}.

The following growth parameters were collected: plant height after 30–60-80 and 100 days from transplanting, branching height and colour of leaves. Leaf colour (L*, a*, and b* parameters - CIELab) was measured using a Colorimeter (Chroma-meter CR-400, Minolta corporation, Ltd., Osaka, Japan) at two points of photosynthetic tissue on the upper side of 20 leaves for each replicate, randomly selected, for all treatments.

Hue angle (h°) and Chroma (C*) were calculated as h° = arctan(b*/a*) when a* > 0 and b* > 0, or as h° = 180° + arctan(b*/a*) when a* < 0 and b > 0 (McGuire, 1992) and $C^* = (a^{*2} + b^{*2})^{1/2}$.

Commercially mature fruits were harvested according to fruit dimension, colour and glossiness. Immediately after harvesting fruits were weighed and marketable and unmarketable yield, number and average weight of marketable fruit were calculated. Yield and number of fruit were calculated per plant. Quality characteristics of eggplant fruit were measured in 10 representative commercially mature fruits for each replicate. Colour (L*, a*, and b* parameters - CIELab) was measured on two opposite points of eggplant fruit skin (equatorial zone) by a tristimulus Minolta Chroma meter CR-400. Chroma (C*) and Hue angle (H°) were also calculated as follows: $C^* = (a^{*2} + b^{*2})^{1/2}$, H° = arctan (b*/a*). The colorimeter was also used to determine the lightness of fruit pulp by measuring L* value (0 = black and 100 = white). Fruits were then sectioned in the equatorial part and the colour of the pulp was measured immediately after cutting (L_0) and after 30 min (L_{30}) in two areas (central and lateral) of the section in order to determine the oxidation potential (Moncada et al., 2013). Pulp lightness were expressed as L_0, while the oxidation potential was expressed as $\Delta L_{30} = (L_0 - L_{30})$.

The firmness of fruit skin and pulp was determined by measuring its resistance to the plunger of a digital penetrometer (Tr snc, Italy). Each fruit was punched in two opposite points of the equatorial part of the skin (using a 6 mm diameter stainless steel cylinder probe) and, after fruit sectioning, a transversal central slice (2 cm high) was punched (8 mm diameter stainless steel cylinder probe) in two points (central and lateral). The mean peak force was calculated in Newtons.

Samples of the fruit pulp were squeezed by hand with a garlic squeezer. The juice was filtered and SSC was measured using a digital refractometer (MTD-045nD, Three-In-One Enterprises Co. Ltd. Taiwan). The dry matter of fruits was measured drying fruits in a thermo-ventilated oven at 80°C for 3 days until constant weight was reached.

Treatments were defined by a completely randomized design with three replicates per treatment, each consisting of 16 plants. Statistical analysises were performed using ANOVA and mean separation was performed by Duncan multiple range test.

Percentages were subjected to angular transformation prior to perform statistical analysis ($\Phi = \arcsin(p/100)^{1/2}$).

7.3 Results and discussion

7.3.1 Grafting success, rooting, grafted cuttings growth, and nursery timeline

Grafting success showed no consistent trend relative to the treatment effects in the two trials (ranging 95 – 100% per treatment, data now shown). Hence, all evaluations regarding growth were carried out for those cuttings successfully taken. All cuttings, and grafted cuttings developed roots at the end of the rooting stage.

Post-propagation growth was significantly affected by the propagation technique (Table 7.1), even though the unrooted grafted cutting (B/St-cutting) did not show statistical significant differences in comparison to grafted seedling (B/St-seedling) (Table 7.1). The highest fresh and dry weight values (canopy and roots), were obtained by traditional grafted seedlings (B/St-seedling) (Table 7.2). While, the lowest dry weight values were showed by plantlets with adventitious roots (B cutting, B/B-cutting, and B/St-cutting). However, considering all treatments, there were not statistical significant differences regarding the percentage of dry mass (Table 7.2). Non-existent differences in dry weight was probably associated with the healing condition, that allowed at least null or positive carbon balance and thereby no loss in dry mass during the healing phase. In fact, Johnson and Miles (2011) demonstrated that healing chamber designs affect the conditions and thereby the grafting success (quantity and quality), even though it appear that further research needs to be done for optimizing healing conditions. The treatment B/St-seedling shows an advantage regarding the roots length, five days after the end of propagation in comparison to others treatments (Table 7.2). Number of true leaves after five days of growth in greenhouse did not show statistical significant differences between all treatments, while, the scion epicotyl length was smallest in the treatment B/B-seedling. However, the eggplant grafted onto *S. torvum* rootstock in both cases, unrooted grafted cutting (B/St-cutting) and grafted seedling (B/St-seedling), did not show statistical significant differences between them, suggesting that the growth and development are not promoted or suppressed by the healing conditions used (mist propagation or humidity chamber).

A great advantage in nursery production time was observed regarding the grafted eggplant plantlets production through unrooted grafted cutting propagation technique (Table 7.3). This technique (B/St-cutting) allowed to obtain finished grafted plantlets within 66 days compared to 84 days required by standard eggplant grafting technique (B/St-seedling), with a good plantlets visual quality (data not shown). Therefore, the great improvement of the nursery grafted eggplant production time, might allow to overcome a national issue concerning the low grafted eggplant seedlings production flexibility due to long-period of production required, and an international issue, specifically in United States, regarding the difficulty of the propagators that can not meet supply of farmers and growers in North America, creating a bottleneck.

7.3.2 Production and morphological data

Although the mean number of fruits per plant was similar for all treatments, irrespective of roots type (adventitious roots or seminal root) and propagation technique (grafted or ungrafted), the fruit yield, concerns: total yield per plant, marketable yield per plant, and average fruit weight, were significantly higher in the grafted plants onto *S. torvum* rootstock. However, there were not statistical significant differences between treatments B/St-seedling and B/St-cutting. This difference in yield was observed in both experiments (Years 1 and 2) and was the result from the greater average fruit weight from grafted plants onto *S. torvum* compared with the others treatments (Tables 7.4 and 7.5).

Even though at 30th day after transplant the plant heights have not shown significant statistical differences, at 60th, 80th, and 100th day from transplant, plants grafted onto *S. torvum* rootstock, have given higher values (Tables 7.6 and 7.7), according to Bletsos (2003). However, in B/St-seedling and B/St-cutting treatments, were not observed significant differences, probably due to similar nutrient and water uptake. This trend in plant height was also observed in branch height (Tables 7.6 and 7.7). The evaluation concerning the leaf colour, showed the highest L*, b*, and Chroma values in B/St-seedling and B/St-cutting treatments, while the same treatments, have given the lowest a* and Tinta values. Therefore, the plants grafted onto *S. torvum* rootstock showed a greater leaf lightness even though with lower greenness (Tables 8 and 9).

7.3.3 Fruit and plant quality

Data on the berry dry matter showed that the self-grafted plants, produced berries characterized by higher values in both experiments (Years 1 and 2). This was due to rootstock effect. Nevertheless, were not found significant differences between plants propagated by standard grafting (B/St-seedling) and those propagated by unrooted grafted cutting (B/St-cutting) (Table 7.8 and 7.9). Skin firmness was in both years highest in fruits from ungrafted plants, while pulp firmness was in both years similar for all treatments as well as the SSC, and the fruit colour values (Table 7.8 and 7.9). The low inner physicochemical variability of the fruits, can be related to many factors, such as the high uniformity of fruits, and the ripening of eggplant fruit not very rapid. These factors may explain very low diversity of fruit firmness, fruit colour, as well as variability of their SSC content between treatments. Although the L_0 (pulp lateral area) values showed significant differences, the ΔL_{30} values have not showed appreciable differences, while regarding the L_0 and ΔL_{30} (pulp central part), in both cases, the propagation technique has not affected these parameters (Table 7.10 and 7.11). The explanation for this may be due to the fact that the pulp oxidation is a character strongly influenced by scion variety, in this case 'Birgah' eggplant**.**

7.4 Conclusions

The propagation success was not affected by adopted techniques. Post-propagation growth was not, however, suppressed in the plantlets propagated by unrooted grafted cutting, despite significant reduction of fresh and dry mass was observed. However, the percentage of dry mass remained similar. Moreover, the productive potential was not altered in quality much less in amount.

Based on our studies, unrooted grafted cuttings propagation technique, using cuttings of *S. torvum* harvested from stock plants, might represent a viable solution to overcome the long-period required to produce grafted eggplant plantlets due to low germination seed of *S. torvum*, and slow growth of the rootstock seedlings. Further studies are under way, concerning the tolerance or resistance to abiotic stresses such as the drought stress of plantlets characterized by adventitious roots such as those under consideration.

Table 7.1 – Growth and developmental parameters of grafted eggplant transplants produced by using un-rooted grafted cuttings

	Grafting stage		End of propagation		After 5 days of growth	
Propagation type	# Leaves	Plantlets total lenght [cm]	# Leaves	Plantlets total lenght [cm]	# Leaves	Plantlets total lenght [cm]
B seedling	4.05 n.s.	7.58 a	5.60 ab	9.23 a	6.25 n.s.	9.38 c
B cutting	4.10 n.s.	6.00 c	5.75 a	6.58 b	6.10 n.s.	7.20 d
B/B-seedling	4.10 n.s.	7.22 ab	5.50 ab	9.18 a	6.30 n.s.	11.50 a
B/B-cutting	4.05 n.s.	6.39 bc	5.09 abc	7.10 b	6.15 n.s.	9.18 c
B/St-seedling	3.85 n.s.	6.62 bc	4.35 c	8.65 a	5.50 n.s.	10.35 b
B/St-cutting	4.05 n.s.	6.75 bc	4.89 bc	8.40 a	5.90 n.s.	10.65 b

In each column. figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 7.2 – Above-ground and roots development of grafted eggplant transplants produced by using un-rooted grafted cuttings

	Canopy			Roots			
Propagation type	Fresh mass [g]	Dry mass [g]	% Dry mass	Fresh mass [g]	Dry mass [g]	% Dry mass	Lenght [cm]
B seedling	2.95 b	0.38 ab	12.71 n.s.	1.52 bc	0.10 b	6.58 bc	5.43 c
B cutting	3.00 b	0.33 bc	10.83 n.s.	1.28 c	0.10 b	7.81 b	6.95 bc
B/B-seedling	3.28 b	0.38 ab	11.43 n.s.	0.80 d	0.10 b	12.50 a	5.25 c
B/B-cutting	3.03 b	0.33 bc	10.73 n.s.	1.78 b	0.10 b	5.62 c	8.55 b
B/St-seedling	3.98 a	0.45 a	11.31 n.s.	2.95 a	0.20 a	6.78 bc	11.53 a
B/St-cutting	2.93 b	0.25 c	8.53 n.s.	1.55 bc	0.10 b	6.45 bc	7.83 bc

In each column. figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 7.3 – Time and phases of eggplant propagation

Propagation type	Sowing of rootstock	Sowing of scion	Graft	Harvesting and rooting	Beginning acclimatization	End of propagation
B seedling	-	0	-	-	-	50
B cutting	-	0	-	50	70	72
B/B-seedling	0	0	50	-	57	59
B/B-cutting	0	0	50	50	70	72
B/St-seedling	0	25	75	-	82	84
B/St-cutting	-	0	50*	50	64	66

* harvesting of rootstock cuttings from stock plants.

Table 7.4 – Effect of eggplant propagation type on yield (first year)

Propagation type	Total yield [kg plant-1]	Unmarketable yield [%]	# of marketable fruits plant^{-1}	Marketable yield [Kg plant^{-1}]	Average fruit weight [g]
B seedling	5.4 ab	22.3 a	10.5 a	4.2 c	394.5 b
B cutting	5.3 ab	23.5 a	10.8 a	4.0 c	373.5 b
B/B-seedling	5.4 ab	15.8 b	11.4 a	4.5 bc	396.7 b
B/B-cutting	5.0 b	14.5 b	11.1 a	4.2 c	382.9 b
B/St-seedling	5.9 a	13.7 b	11.8 a	5.1 a	430.4 a
B/St-cutting	5,8 a	12.1 b	11.6 a	5.1 a	442.4 a

In each column. figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 7.5 – Effect of eggplant propagation type on yield (second year)

Propagation type	Total yield [kg plant-1]	Unmarketable yield [%]	# of marketable fruits plant^{-1}	Marketable yield [Kg plant^{-1}]	Average fruit weight [g]
B seedling	4.5 ab	11.9 a	10.3 a	3.8 ab	388.4 ab
B cutting	4.0 b	15.9 a	8.9 a	3.3 ab	354.9 b
B/B-seedling	6.1 b	18.4 a	1.9 a	4.0 b	370.2 b
B/B-cutting	6.2 b	14.9 a	12.1 a	5.2 b	371.4 b
B/St-seedling	5.3 a	11.6 a	12.1 a	4.7 a	435.3 a
B/St-cutting	5.0 a	15.7 a	11.9 a	4.2 a	431.0 a

In each column. figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 7.6 – Effect of eggplant propagation type on plant growth (first year)

	Plant height [cm]				Branch height [cm]	
Propagation type	30 d	60 d	80 d	100 d	30 d	60 d
B seedling	64.0 a	96.2 bc	117.0 b	119.3 b	23.5 a	25.1 a
B cutting	61.6 a	90.0 c	116.1 b	117.3 b	20.7 b	23.6 b
B/B-seedling	57.5 a	94.2 bc	115.7 b	121.6 b	17.7 c	20.2 c
B/B-cutting	59.9 a	89.6 c	117.3 b	122.0 b	23.9 a	26.4 a
B/St-seedling	64.0 a	100.8 ab	134.4 a	135.7 ab	19.5 b	21.9 bc
B/St-cutting	61.8 a	108.2 a	141.5 a	139.7 a	20.6 b	22.2 b

In each column. figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 7.7 – Effect of eggplant propagation type on plant growth (second year)

Propagation type	Plant height [cm]				Branch height [cm]	
	30 d	60 d	80 d	100 d	30 d	60 d
B seedling	28.0 a	63.6 b	83.9 b	107.7 b	13.2 ab	26.4 b
B cutting	25.6 a	61.0 b	84.66 b	104.3 b	13.1 bc	22.5 b
B/B-seedling	25.0 a	55.8 b	75.1 b	98.6 bc	13.8 ab	25.0 b
B/B-cutting	24.7 a	56.8 b	77.7 b	89.1 c	13.5 ab	23.3 b
B/St-seedling	31.0 a	80.6 a	110.5 a	133.7 a	15.4 ab	35.7 a
B/St-cutting	31.5 a	81.1 a	107.0 a	135.1 a	16.0 a	35.3 a

In each column. figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 7.8 – Effect of eggplant propagation type on qualitative parametres (first year)

Propagation type	Dry matter	Firmness [N]		SSC [°Brix]	Skin colour				
		Skin	Pulp		L*	a*	b*	Chroma	Hue angle
B seedling	6,1 b	38,6 ab	17,1 a	4,1 a	26,3 a	12,0 a	-3,7 a	12,6 a	343,2 ab
B cutting	6,0 b	38,1 ab	18,0 a	4,0 a	26,1 a	10,9 a	-3,2 a	11,3 a	343,8 ab
B/B-seedling	7,2 a	45,1 a	19,5 a	4,1 a	25,8 a	10,8 a	-3,4 a	11,4 a	342,5 b
B/B-cutting	7,3 a	44,7 a	17,7 a	4,2 a	25,9 a	10,2 a	-3,1 a	10,7 a	343,2 ab
B/St-seedling	6,2 b	33,6 b	18,1 a	3,7 a	25,6 a	11,0 a	-3,1 a	11,4 a	344,7 a
B/St-cutting	5,8 b	33,0 b	17,4 a	3,7 a	25,3 a	11,9 a	-3,4 a	12,3 a	344,2 a

In each column. figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 7.9 – Effect of eggplant propagation type on qualitative parametres (second year)

Propagation type	Dry matter	Firmness [N]		SSC [°Brix]	Skin colour				
		Skin	Pulp		L*	a*	b*	Chroma	Hue angle
B seedling	6,0 b	36,4 a	17,9 a	3,1 a	26,8 b	13,8 b	-4,6 a	14,6 cd	341,6 b
B cutting	5,9 b	35,9 a	17,4 a	3,5 a	28,5 a	16,3 a	-6,4 b	17,5 a	338,7 d
B/B-seedling	7,1 a	25,6 b	16,7 a	3,1 a	26,9 b	16,0 a	-6,0 b	17,1 ab	339,4 cd
B/B-cutting	7,1 a	28,2 b	15,7 a	3,0 a	27,3 ab	14,6 b	-5,3 ab	15,5 b	340,2 c
B/St-seedling	6,2 b	23,4 b	15,3 a	3,0 a	26,6 b	14,5 b	-4,4 a	15,1 c	343,1 a
B/St-cutting	6,0 b	28,0 b	15,4 a	3,0 a	26,0 b	12,3 c	-4,1 a	13,0 d	341,7 b

In each column. figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 7.10 – Effect of eggplant propagation type on oxidation potential (first year)

	Pulp central area		**Pulp lateral area**	
Propagation type	**L_0**	**ΔL_{30}**	**L_0**	**ΔL_{30}**
B seedling	90,9 a	-2,2 a	88,4 ab	-1,1 a
B cutting	90,5 a	-2,2 a	87,9 b	-1,2 a
B/B-seedling	90,7 a	-1,8 a	88,9 ab	-2,3 ab
B/B-cutting	90,6 a	-1,9 a	89,1 a	-2,8 b
B/St-seedling	90,5 a	-1,9 a	88,6 ab	-2,2 ab
B/St-cutting	90,7 a	-1,6 a	87,5 b	-1,0 a

In each column. figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

Table 7.11 – Effect of eggplant propagation type on oxidation potential (second year)

	Pulp central area		Pulp lateral area	
Propagation type	L_0	ΔL_{30}	L_0	ΔL_{30}
B seedling	88,6 a	-0,1 a	90,5 b	-4,1 a
B cutting	89,5 a	-0,4 a	90,2 b	-2,3 a
B/B-seedling	90,9 a	-0,7 a	91,4 ab	-4,3 a
B/B-cutting	90,0 a	-0,3 a	90,7 a	-2,2 a
B/St-seedling	89,4 a	-0,2 a	89,8 bc	-3,1 a
B/St-cutting	89,0 a	-0,9 a	89,2 c	-3,2 a

In each column. figures followed by the same letter were found to be not statistically different, based on the Duncan test ($P \leq 0.05$).

8
Concluding remarks

Grafting is a propagation technique which involves complexes biological processes. This study highlighted the importance of grafting in the exploitation of local eggplant populations. The production of high quality vegetables is often linked to the use of local ecotypes. Therefore, the recovery, characterization and diffusion of old, native populations are not the start of agricultural and cultural regression, but rather the chance to help face ecological issues and those concerning agro-ecosystem sustainability.

In addition, the grafting techniques in eggplant, allows a reduction of some metals, such as Na and Mn. This variation could be of significant interest as lower levels favour a reduction in hypertension and help keep blood pressure under control. Regarding the possibility to improve the antioxidant content through the grafting in eggplant, the results indicate that the rootstock does not substantially improve the phenolic metabolism, but the improvement of grafting is strictly related to scion-rootstock combination. Furthermore, the graft has proved a useful technique to increase the productive potential, especially as regard the increasing number of marketable fruits. Therefore, the answer to the question whether grafting always improves the quantitative and qualitative yields seems to be: for quantitative properties, yes; for qualitative properties, no.

Finally, unrooted grafted cutting proved to be a suitable propagation technique to solve problems correlated to the long-term required to produce grafted eggplant seedlings using *S. torvum* as rootstock, as well as those related to the high transport cost of watermelon grafted seedlings in United States.

References

Alan, O.; Ozdemir, N.; Gunen, Y. Effect of grafting on watermelon plant growth, yield and quality. J. Agron. 2007, 6, 362–365.

An, Y., Kim, Y., Kwon, T., Jeong, S., 2004. Combined effect of copper, cadmium, and lead upon Cucumis sativus growth and bioaccumulation. Sci. Total Environ. 326, 85–93.

Angelova, V.R., Babrikov, T.D., Ivanov, K.I., 2009. Bioaccumulation and distribution of lead, zinc, and cadmium in crops of Solanaceae family. Commun. Soil Sci. Plant Anal. 40, 2248–2263.

Arao, T., Takeda, H., Nishihara, E., 2008. Reduction of cadmium translocation from roots to shoots in eggplant (Solanum melongena) by grafting onto Solanum torvum rootstock. Soil Sci. Plant Nutr. 54, 555–559.

Ashita, E. (Ed.), 1927. Grafting of watermelons. Korea (Chosun) Agricultural Newsletter 1, 9 (in Japanese).

Babcock B.A. and Clemens R. 2004. Geographical indications and property rights: protecting value-added agricultural products.*MATIC Briefing Paper 04-MBP 7*. Iowa State University. IA, 51 pp.

Bletsos, F., Thanassoulopoulos, C. and Roupakias, D. 2003. Effect of grafting on growth, yield and Verticillium wilt of eggplant. HortScience 38:183-186.

Bletsos, F.A., 2005. Use of grafting and calcium cyanamide as alternatives to methyl bromide soil fumigation and their effects on growth, yield, quality and Fusarium wilt control in melon. J. Phytopathol. **153**:155–161.

Cao G., Sofic. E., Prior R.L. 1996. Antioxidant capacity of tea and common vegetables. Journal of Agricultural and Food Chemistry **44**. 3426–3431.

Clemens, S., 2001. Molecular mechanisms of plant metal tolerance and homeostasis. Planta 212, 475–486.

Clemens, S., 2006. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie 88, 1707–1719.

Colla, G., Rouphael, Y., Cardarelli, M., Rea, E., 2006. Effect of salinity on yield, fruit quality, leaf gas exchange, and mineral composition of grafted watermelon plants, Hort. Sci., **41**: 622-627.

Colla, G., Rouphael, Y., Cardarelli, M.,Massa, D., Salerno, A., Rea, E., 2006a. Yield, fruit quality and mineral composition of grafted melon plants grown under saline conditions. J. Hortic. Sci. Biotechnol. 81, 146–152.

Colla, G., Rouphael, Y., Leonardi, C., Bie, Z. 2010. Role of grafting in vegetable crops grown under saline conditions. Scientia Horticulturae 127, 147–155

Colla, G., Suãrez, C.M.C., Cardarelli, M., Rouphael, Y., 2010a. Improving nitrogen use efficiency in melon by grafting. HortScience 45, 559–565.

Colla, G., Rouphael, Y., Cardarelli, M., Salerno, A., Rea, E., 2010b. The effectiveness of grafting to improve alkalinity tolerance in watermelon. Environ. Exp. Bot. 68, 283–291.

Demirsoy, H.; Macit, I. Dwarfing mechanism in fruit trees. J. Fac. Agric. OMU 2007, 22, 214–218.

D'Anna, F., Sabatino, L., 2013. Morphological and agronomical characterization of eggplant genetic resources from the Sicily area. Journal of Food, Agriculture and Environment. Journal of Food, Agriculture & Environment, Vol. **11** (1): 401-404.

Dauchet L, Amouyel P, Dallongeville J. (**2009**) Fruits, vegetables and coronary heart disease. *Nature Reviews Cardiology*, ***6***, 599-608

Daunay, M.C., 2008. Eggplant. In: Prohens, J., Nuez, F. (Eds.), Handbook of Plant Breeding: Vegetables II. Springer, New York, USA, pp. 163–220.

Daunay M.C., Aubert S., Frary A., Doganlar S., Lester R.N., Barendse G., van der Weerden G., Hennart J.W., Haanstra J., Dauphin F., Jullian E. 2004. Eggplant (*Solanum melongena* L.) fruit colour: pigments. measurements and genetics. In Proceedings XIIth Meeting on Genetics and Breeding of Capsicum & Eggplant. pp. 108–116. Eds R.E. Voorrips. Noordwijkerhout. the Netherlands: Plant Research International.

Dave YS, Patel ND, Rao KS. (1979) The study of origin of pericarp layers in Solanum melongena. Phyton (Austria), 19, 233-241.

Davis, A.R., Perkins-Veazie, P., Hassell, R., Levi, A., King, S.R., Zhang, X.P., 2008a. Grafting effects on vegetable quality. HortScience **43**:1670–1672.

Davis, A.R., Perkins-Veazie, P., Sakata, Y., López-Galarza, S., Maroto, J.V., Lee, S.G., Huh, Y.C., Sun, Z., Miguel, A., King, S., Cohen, R., Lee, J.M., 2008b. Cucurbit grafting. Crit. Rev. Plant Sci. **27**:50–74.

Devanand Luthria, Ajay P. Singh, Ted Wilson, Nicholi Vorsa, Gary S. Banuelos, Bryan T. Vinyard. 2010. Influence of conventional and organic agricultural practices on the phenolic content in eggplant pulp: Plant-to-plant variation. Food Chemistry Vol. 121 406–411

Diánez, F., Díaz, M., Santos, M., Huitrón, V., Ricárdez, M., Camacho, F. (2007). The use of grafting in Spain. Proceedings of Technical Meeting on Non-Chemical Alternatives for Soil-Borne Pest Control, Budapest: 87-97.

Dyki, B., Jankiewicz, L.S. & Staniaszek, M. 1997. Anatomy and surface micromorphology of Tomatillo fruit (Physalis ixocarpa Brot.). Acta Societatis Botanicorum Poloniae 66: 21-27.

Dong, H.H., Niu, Y.H., Li, W.J., Zhang, D.M., 2008. Effects of cotton rootstock on endogenous cytokinins and abscisic acid in xylem sap and leaves in relation to leaf senescence. J. Exp. Bot. 59, 1295–1304.

Edelstein, M., Ben-Hur, M., Cohen, R., Burger, Y., Ravina, I., 2005. Boron and salinity effects on grafted and non-grafted melon plants. Plant Soil 269, 273–284.

Edelstein, M., Ben-Hur, M., 2007. Preventing contamination of supply chains by using grafted plants under irrigation with marginal water. In: Wilson, J. (Ed.), Proceedings of the International Symposium on Water Resources Management. Honolulu, Hawaii, USA, pp. 150–154.

Edelstein, M., Ben-Hur, M., Plaut, Z., 2007a. Grafted melons irrigated with fresh or effluent water tolerate excess boron. J. Am. Soc. Hortic. Sci. 132, 484–491.

Eva Sánchez-Rodríguez et al., 2011. Phenolic Metabolism in Grafted versus Nongrafted Cherry Tomatoes under the Influence of Water Stress. Journal of Agriculture and Food Chemistry. Vol. 59, 8839–8846.

Flick, G.J., Burnette, F.S., Aung, L.H., Ory, R.L., St. Angelo, A., 1978. Chemical composition and biochemical properties of mirlitons (Sechium edule) and purple, green, and white eggplants (Solanum melongena). Journal of Agricultural and Food Chemistry **26**:1000–1005.

Fogg.D.N., Wilkinson N.T., 1958. The colorimetric determination of phosphorus: The Analist **83**:406-414.

Garibaldi. A. and Minuto. A. (2003). The application of grafting against soilborne pests and diseases of tomato in Italy: present situation and perspectives. Procedings of International congress "Greenhouse tomato - Integrated crop production - Organic production", Avignon – France:55–59.

Gratão, P.L., Polle, A., Lea, P.J., Azevedo, R.A., 2005. Making the life of heavy metal stressed plants a little easier. Funct. Plant Biol. 32, 481–494.

Hanson, P.M., Yang, R.Y., Tsou, S.C.S., Ledesma, D., Engle, L., Lee, T.C., 2006. Diversity in eggplant (Solanum melongena) for superoxide scavenging activity, total phenolics, and ascorbic acid. Journal of Food Composition and Analysis **19**:594–600.

Hassell, R.L.,and Memmott, F. 2008. Grafting Methods for Watermelon Production. HortScience 43(6) 1677-1679.

Hong-Bo, S., Li-Ye, C., Cheng-Jiang, R., Hua, L., Dong-Gang, G., Wei-Xiang, L., 2010. Understanding molecular mechanisms for improving phytoremediation of heavy metal-contaminated soils. Crit. Rev. Biotechnol. 30, 23–30.

Huang, Y., Zhu, J., Zhen, A., Chen, L., Bie, Z.L., 2009a. Organic and inorganic solutes accumulation in the leaves and roots of grafted and ungrafted cucumber plants in response to NaCl stress. J. Food Agric. Environ. 7, 703–708.

International Official Methods of Analysis, AOAC, 1985a, 1985b, 1985c,)

International Official Methods of Analysis (AOAC) 22.026, 1985a, 22, 362.

International Official Methods of Analysis (AOAC) 3.140, 1985b, 3, 50.

International Official Methods of Analysis (AOAC) 14.018, 1985c, 3, 69.

Jensen WA. (1962) Botanical histochemistry principles and practice. San Francisco, London. WH Freeman and Co.

Jung-Myung Lee, 1994. Cultivation of Grafted Vegetables I. Current Status, Grafting Methods, and Benefits. HORTSCIENCE, VOL. 29(4) 235-239.

Jung-Myung Leea, C. Kubotab, S.J. Tsaoc, Z. Bied, P. Hoyos Echevarriae, L. Morraf, M. Odag. 2010. Current status of vegetable grafting: Diffusion, grafting techniques, automation. Scientia Horticulturae. Vol. 127 93–105

Kato, T., Lou, H., 1989. Effect of rootstock on the yield, mineral nutrition and hormone level in xylem sap in eggplant. J. Jpn. Soc. Hortic. Sci. 58, 345–352.

King, S.R., Davis, A.R., Liu, W., Levi, A., 2008. Grafting for disease resistance. HortScience **43**:1673–1676.

King, S.R., Davis, A.R., Zhang, X., Crosby, K., 2010. Genetics, breeding and selection of rootstocks for Solanaceae and Cucurbitaceae. Sci. Hortic. **127**:106–111.

Klemt, F. 1907. Über den bau und die Entwicklung einiger Solanaceenfrüchte. Inaugural Diss., 1-35. Berlin

Ko, K.D., 1999. Response of cucurbitaceous rootstock species to biological and environmental stresses. Ph.D. Diss. Seoul Nat'l Univ., Korea.

Ko, K.D., 2008. Current status of vegetable seedling production in Korea and its prospects. In: Inauguration Seminar of Korean Plug Growers Assoc., June.

Kubota, C., McClure, M.A., Kokalis-Burelle, N., Bausher, M.G., Rosskopf, E.N., 2008. Vegetable grafting: history, use and current technology status in North America. HortScience 43, 1663–1669.

La Malfa G. 1990. Scienza e tecniche delle produzioni vegetali. Orticoltura. pp. 793-811.

Lee, J.M., 1994. Cultivation of grafted vegetables I: current status, grafting methods and benefits. HortScience **29**:235–239.

Lee, J.M., Oda, M., 2003. Grafting of herbaceous vegetable and ornamental crops. Hort. Rev., **28**: 61-124.

Lee, J.M., Kubota, C., Tsao, S.J., Vinh, N.Q., Huang, Y., Oda, M., 2008. Recent progress in vegetable grafting. In: International Workshop on Development and Adoptation of Green Technology for Sustainable Agriculture and Enhancement of Rural Entrepreurship, IRRI, Los Banós, Laguna, Philippines, September 28–October 2, 2009, 21 pp.

Lee, J.M., Kubota, C., Tsao, S.J., Vinh, N.Q., Huang, Y., Oda, M., 2010. Current status of vegetable grafting: Diffusion, grafting techniques, automation. Scientia Horticulturae **127** 93–105.

Ling-Lee M, Chilvers GA, Ashford AE. (1977) A histochemical study of phenolic materials in mycorrizhal and uninfected roots of Eucalyptus festigata, Dean and maiden. New Phytologist, 78, 313-328.

Liu, H.P., Dong, B.H., Zhang, Y.Y., Liu, Z.P., Liu, Y.L., 2004. Relationship between osmotic stress and the levels of free, conjugated and bound polyamines in leale of wheat seedlings. Plant Sci. 166, 1261–1267.

Masanao, U. and Y. Hisaya. 1996. Development of full automatic grafting robot for fruit vegetables. Robot Tokyo. 109:59–65.

McDowell, L.R., 2003. Minerals in Animal and Human Nutrition. Elsevier Science Health Sciences, Amsterdam, The Netherlands.

Miguel, A., Maroto, J.V., San Bautista, A., Baixauli, C., Cebolla, V., Pascual, B., López, S., Guardiola, J.L., 2004. The grafting of triploid watermelon is an

advantageous alternative to soil fumigation by methyl bromide for control of Fusarium wilt. Sci. Hortic. **103**:9–17.

Miller, R.H. 1969. A morphological study of Solanum mammosum and its mammiform fruit. Botanical Gazette 130: 230-237.

Minuto, A., Serges, T., Nicotra, G., and Garibaldi, A. (2007). Applicazione dell'innesto erbaceo per le solanacee allevate in coltura protetta: problematiche e prospettive. Informatore fitopatologico – La difesa delle piante, 57:30–36.

Moncada, A., Miceli, A., Vetrano, F., Mineo, V., Planeta, D., D'Anna, F., 2013. Effect of grafting on yield and quality of eggplant (Solanum melongena L.). Sci. Hortic. **149**:108-114.

Morand P.,Gullo J.L., 1970. Mineralisation des tissus vegetaux en vue du dosage de P,Ca,Mg, Na,K Ann.Agron, **21** (2):229,236.

Morra, L., Bilotto, M., 2009. Mercato in fortissima ascesa per i portainnesti orticoli. L'Informatore Agrario 1, 51–54.

Moustakas, N.K., Akoumianakis, K.A., Passam, H.C., 2001. Cadmium accumulation and its effect on yield of lettuce, radish and cucumber. Commun. Soil Sci. Plant Anal. 32, 1793–1802.

Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59, 651–681.

Nisini P., Colla G., Granati E., Temperini O., Crino P., Saccardo F., 2002. Rootstock resistance to Fusarium wilt and effect on fruit yield and quality of two muskmelon cultivars. Scientia Horticulturae **93**:281-288.

Oda, M., Nagata, M., Tsuji, K., Sasaki, H., 1996. Effects of scarlet eggplant rootstock on growth, yield, and sugar content of grafted tomato fruits. J. Jpn. Soc. Hortic. Sci. **65**:531–536.

Oshiro, N., Kuniyoshi, K., Nakamura, A., Araki, Y., Tamanaha, K., Inafuku, Y., 2008. A case of food poisoning due to ingestion of eggplant, Solanum melongena, grafted on devil's trumpet, Datura metel [in Japanese]. Shokuhin Eiseigaku Zasshi **49**:376–379.

Osteen C. (**2003**) Methyl bromide phase-out proceeds: users request exemptions USDA. *Amber Waves*, ***1***, 23–27.

Otani, T., Seike, N., 2006. Comparative effects of rootstock and scion on diedri and endrin uptake by grafted cucumber (Cucumis sativus). J. Pestic. Sci. 31, 316–321.

Otani, T., Seike, N., 2007. Rootstock control of fruit dieldrin concentration in grafted cucumber (Cucumis sativus). J. Pestic. Sci. 32, 235–242.

Pearse Everson AG. (1961) Histochemistry theoretical and applied, J. e A. Churchill Ltd, London.

Piergiovanni A.R. and Laghetti G. 1999. The common bean landraces from Basilicata (southern Italy): an example of integrated approach applied to genetic resources management. *Genet Res Crop Evol* **46**:47–52.

Proietti, S., Rouphael, Y., Colla, G., Cardrelli, M., de Agazio, M., Zacchini, M., Rea, E., Moscatello, S., Battistelli, A., 2008. Fruit quality of mini-watermelon as affected by grafting and irrigation regimes. J. Sci. Food. Agric. **88**:1107–1114.

Prohens J., Nuez F. 2001. Variedades tradicionales de berenjena en España. Vida Rural. **130**. 46–50.

Raimondo F. M., Gianguzzi L., Ilardi V. 1992. Inventario delle specie "a rischio" nella flora vascolare della Sicilia. Quaderni di botanica ambientale applicata. **3**: 65-132.

Rao R., Corrado G., Bianchi M., Di Mauro A. and (GATA)4. 2006. DNA fingerprinting identifies morphologically characterized 'San Marzano' tomato plants. *Plant Breed* **125**:173–176

Raskin, I., Smith, R.D., Salt, D.E., 1997. Phytoremediation of metals: using plants to remove pollutants from the environment. Curr. Opin. Biotechnol. 8, 221–226.

Ristanio J, Thomas W. (**1997**) Agriculture, methyl bromide, and the ozone hole, can we fill the gaps. *Plant Disease*, ***81***, 964–976.

Rivero, R. M., Ruiz, J. M., & Romero, L. 2003a. Role of grafting in horticultural plants under stress conditions. Journal of Food Agriculture and Environment 1(1):70-74.Shetty

Rivero, R.M., Ruiz, J.M., Sanchez, E., Romero, L., 2003b. Does grafting provide tomato plants an advantage against H2O2 production under conditions of thermal shock? Physiol. Plant. 117, 44–50.

Romero, L., Belakbir, A., Ragala, L., Ruiz, J.M., 1997. Response of plant yield and leaf pigments to saline conditions: effectiveness of different rootstocks in melon plants (Cucumis melo L.). Soil Sci. Plant Nutr. 43, 855–862.

Rouphael, Y., Cardarelli, M., Colla, G., Rea, E., 2008a. Yield, mineral composition, water relations, and water use efficiency of grafted mini-watermelon plants under deficit irrigation. HortScience 43, 730–736.

Rouphael, Y., Cardarelli, M., Colla, G., 2008. Grafting of cucumber as a means to minimize copper toxicity. Environ. Exp. Bot., **63**: 49-58.

Rubatzky, V.E., Yamaguchi, M., 1997. World Vegetables: Principles, Production and Nutritive Values. Chapman & Hall, New York, USA.

Russo, V.M., 1996. Cultural methods and mineral content of eggplant (Solanum melongena) fruit. Journal of the Science of Food and Agriculture **71**:119–123.

Sabatino, L., Palazzolo, E., D'Anna, F. 2013. Grafting suitability of Sicilian eggplant ecotypes onto Solanum torvum: Fruit composition, production and phenology. Journal of Food, Agriculture & Environment Vol.11 (3&4): 1195-1200.

Santa-Cruz, M.M., Martinez-Rodriguez, F., Perez-Alfocea, R., Romero-Aranda, R., Bolarin, M.C., 2002. The rootstock effect on the tomato salinity response depends on the shoot genotype. Plant Sci. 162, 825–831.

Sakata, Y., Ohara, T., Sugiyama, M., 2007. The history and present state of the grafting of cucurbitaceous vegetables in Japan. Acta Hortic. 731, 159–170.

Savin F. 1996. L'aubergine dans le bassin méditerranéen (hors Turquie). PHM Revue Horticole. **374**. 50–52.

Savvas, D., Lenz, F., 1996. Influence of NaCl concentration in the nutrient solution on mineral composition of eggplants grown in sand culture. Angewandte Botanik **70**:124–127.

Savvas, D., Papastavrou, D., Ntatsi, G., Ropokis, A., Olympios, C., Hartmann, H., Schwarz, D., 2009. Interactive effects of grafting and Mn-supply level on growth, yield and nutrient uptake by tomato. HortScience 44, 1978–1982.

Schiavi M., Masera R., Lorenzoni C., Soressi G.P. 1991. Metodi utilizzati per il miglioramento genetico della Dorata di Parma. Agricoltura Ricerca n. 82, 21-30 pp. Ismea. Roma.

Schippmann U., Leaman D.J., Cunningham A.B. 2002. Impact of cultivation and gathering of Medicinal Plants on biodiversity: global trends and issues. "Biodiversity and the ecosystem approach in Agriculture. Foresty and Fisheries". FAO. Rome: 21 pp.

Si, Y., Dane, F., Rashotte, A., Kang, K., Singh, N.K., 2010. Cloning and expression analysis of the Ccrboh gene encoding respiratory burst oxidase in Citrullus colocynthis and grafting onto Citrullus lanatus (watermelon). J. Exp. Bot. 61, 1635–1642.

Singh, P.K., Gopalakrishnan, T.R., 1997. Grafting for wilt resistance and productivity in brinjal (Solanum melongena L.). Hortic. J. **10**:57–61

Singh AP, Luthria D, Wilson T, Vorsa N, Singh V, Banuelos GS, Pasakdee S. (**2009)** Polyphenols content and antioxidant capacity of eggplant pulp. *Food Chemistry*, ***114***, 955-961**.**

Steinmetz KA, Potter JD. (1996) Vegetables, Fruit, and Cancer Prevention: A Review. Journal of the American Dietetic Association, 37, 1027-1039.

Stommel J.R., Whitaker B.D., 2003. Phenolic acid composition of eggplant fruit in a germplasm core subset. Journal of the American Society for Horticultural Science **128**: 704–710.

Sugiyama, M., Ae, N., Arao, T., 2007. Role of roots in differences in seed cadmium concentration among soybean cultivars—proof by grafting experiment. Plant Soil 295, 1–11.

Suzuki, M., S. Sasaya, and K. Kobayashi. 1998. Present status of vegetable grafting systems. Japan Agr. Res. Quarterly 32:105–112.

Timberlake C. F. 1981. Anthocyanins in fruit and vegetables. In J. Friend. & M. J. C. Rhodes (Eds). Recent advances in the biochemistry of fruit and vegetables (pp. 221-247). New York: Academic Press.

Tiwari A, Jadon RS, Tiwari P, Nayak S. (2009) Phytochemical investigations of crown of Solanum melongena fruit. International Journal of Phytomedicine, 1, 9-11.
Todaro A, Comino F, Rapisarda P, Catalano A, Barbagallo RN, Spagna G. (**2009**) Recovery of anthocyanins from eggplant peel. *Food Chemistry*, ***114***, 434-439.

Traka-Mavrona, E., Koutsika-Sotiriou, M., and Pritsa, T. (2000). Response of squash (*Cucurbita* spp.) as rootstock for melon (*Cucumis melo* L.). Scientia Horticulturae 83:353-362.

UNEP (United Nations Environment Programme), 2006. Handbook for the Montreal Protocol on Substance Protection of the Ozone Layer & the Montreal Protocol on Substances that Deplete the Ozone Layer.

Venema, J.H., Dijk, B.E., Bax, J.M., van Hasselt, P.R., Elzenga, J.T.M., 2008. Grafting tomato (Solanum lycopersicum) onto the rootstock of a high-altitude accession of Solanum habrochaites improves suboptimal-temperature tolerance. Environ. Exp. Bot. 63, 359–367.

Verkleij, J.A.C., Golan-Goldhirsh, A., Antosiewisz, D.M., Schwitzguébel, J.-P., Schröder, P., 2009. Dualities in plant tolerance to pollutants and their uptake and translocation to the upper plant parts. Environ. Exp. Bot. 67, 10–22.

Vinson J. A., Hao Y., Su. X., Zubik L. 1998. Phenol antioxidant quantity and quality in food: vegetables. Journal of Agricultural and Food Chemistry **46**: 3630. 3634.

Wardlaw I.F. 1990. The control of carbon partitioning in plants. New Phytologist. **27**: 341-381.

Wilson CA, Peterson CA. (1983) Chemical composition of the epidermal, hypodermal, endodermal and intervening cortical cell walls of various plants root. Annals of Botany, 51, 759-769.

Yang, L.F., Zhu, Y.L., Hu, C.M., Liu, Z.L., Zhang, G.W., 2006. Effects of NaCl stress on the contents of the substances regulating membrane lipid oxidation and osmosis and photosynthetic characteristics of grafted cucumber. Acta Bot. Boreal-Occident. Sin. 26, 1195–1200.

Yamaguchi, H., Fukuoka, H., Arao, T., Ohyama, A., Nunome, T., Miyatake, K., Negoro, S., 2010. Gene expression analysis in cadmium-stressed roots of a low cadmium accumulating solanaceous plant, Solanum torvum. J. Exp. Bot. 61, 423–437.

Yetisir, H., Caliskan, M.E., Soylu, S., Sakar, M., 2006. Some physiological and growth responses of watermelon [Citrullus lanatus (Thunb.) Matsum. and Nakai] grafted onto Lagenaria siceraria to flooding. Environ. Exp. Bot. 58, 1–8.

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