Journal of Integrative Agriculture 2016, 15(5): 1017–1024

Available online at www.sciencedirect.com

ScienceDirect

RESEARCH ARTICLE

Grafting affects yield and phenolic profile of *Solanum melongena* L. landraces

L Sabatino[1], G Iapichino[1], A Maggio[2], E D' Anna[3], M Bruno[2], F D' Anna[1]

[1] *Department of Agricultural and Forest Sciences, University of Palermo, Palermo 90128, Italy*
[2] *Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo 90128, Italy*
[3] *Regional Centres for Agricultural Technologies (Ce.R.T.A.), University of Foggia, Foggia 71122, Italy*

Abstract

The influence of grafting on agronomical and qualitative characteristics of four Sicilian eggplant landraces was investigated. Grafted and ungrafted plants were compared in the open field in the northern coast of Sicily. *Solanum torvum* seedlings were used as rootstock. Regardless of genotypes tested, grafting significantly increased total fruit production, marketable production, and number of marketable fruits, but did not affect weight of marketable fruits and waste production. Landrace 2 (Sciacca), with black epidermal tissue and pyriform fruit shape, when grafted onto *S. torvum* not only gave a higher yield performance than ungrafted plants, but also showed a higher phenolic antioxidant content. Landrace 4 (Sicilia), with black epidermal tissue and small cylindrical fruits also benefited, when grafted onto *S. torvum*, from a substantial increase in antioxidant fruit content. As consumers' demand for fruits and vegetables rich in compounds important for human health is steadily increasing, these landrace/rootstock combinations should deserve more attention by plant nurseries involved in grafted seedling production and interested in the valorization and conservation of eggplant biodiversity.

Keywords: eggplant, propagation technique, yield, polyphenols, HPLC analysis

1. Introduction

Sicily, the largest Mediterranean island located in southern Italy, is a cultural and a commercial port and one 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 maintained them as local populations and landraces. For this reason, it was estimated a presence of 2 650 taxa (Raimondo *et al.* 1992) in Sicily on an extension of 26 000 km^2. By selecting to local pedoclimatic requirements, farmers obtained a large intra- and inter-specific variability perfectly adapted to the local agricultural environments and displaying particular characteristics, including organoleptic ones (Schiavi *et al.* 1991). Modern breeding depends on the availability of genetic variability which was enlarged by the farmers along the times (Schippmann *et al.* 2002). Consumption of fruits and vegetables is associated with lower incidence and lower mortality rates of cancer in several human cohort and case-control studies (Steinmetz and Potter 1996), as well as with a decrease in blood pressure (Dauchet *et al.* 2009). The health protective effect of fruits and vegetables is attributed to various antioxidants they contain, especially vitamins and phenolic compounds (Kaur and Kapoor 2001).

Received 8 June, 2015 Accepted 22 February, 2016
Correspondence G Iapichino, E-mail: giovanni.iapichino@unipa.it; Tel: +39-9123862215; Fax: +39-9123867519

doi: 10.1016/S2095-3119(15)61323-5

Solanum melongena L., commonly known as aubergine, eggplant, melanzana, garden egg, brinjal, and patlican, produces fruits widely consumed in various parts of the world. Cao *et al.* (1996) ranked eggplant amongst the top ten vegetables in term of oxygen radical absorbance capacity due to its phenolic compounds. More recently, Huang *et al.* (2004) reported the antioxidant activity of various vegetables including eggplant. Several studies have showed that the quantity and quality of phenolic compounds present in eggplant is significantly influenced by genotype (Stommel and Whitaker 2003; Raigon *et al.* 2008), environment and soil type (Savvas and Lenz 1996; Hanson *et al.* 2006), storage conditions (Concellon *et al.* 2004; Luthria and Mukhopadhyay 2006), cultivation systems (Singh *et al.* 2009; Todaro *et al.* 2009; Raigon *et al.* 2010).

Due to recent policy environmental regulations, vegetable grafting is considered a feasible alternative for control of soilborne pathogens. *Solanum torvum* Sw. is one of the recommended rootstocks for eggplant as it confers tolerance to a wide range of telluric pathogens (*Verticillium dahliae* Klebahn, *Ralstonia solanacearum* (Smith), *Fusarium oxysporum* (Schlechtend: Br) f. sp. *melongenae* Matuo and Ishigami, and *Meloidogyne* spp. root-knot nematodes) (Singh and Gopalakrishnan 1997; Bletsos *et al.* 2003; Daunay *et al.* 2008; King *et al.* 2010).

As the demand for eggplant grafted plug plants is growing rapidly, more research is being focused on the effects of the rootstock/scion combination on plant performance in terms of yield and fruit quality. According to Gisbert *et al.* (2011) and Moncada *et al.* (2013) grafting can also influence eggplant phenolic fruit content. However, these authors provided no information on the influence of grafting on individual phenolic concentrations. Maršič *et al.* (2014) report changes of a wide range of phenolics in the fruits of 3 commercial eggplant varieties and one landrace grafted on tomato rootstock. However, their results were inconsistent mostly due to changes in environmental trial conditions. The aim of this study is to evaluate the influence of grafting on agronomical and qualitative characteristics of four eggplant landraces cultivated in Sicily.

2. Results

Regardless of the landraces (Landrace Bianca L1, Sciacca L2, Marsala L3 and Sicilia L4) tested, grafting significantly increased total fruit production, marketable production, and number of marketable fruits (Table 1), but did not modify average weight of marketable fruits and waste production. The higher marketable production of grafted plants (6.6 kg m^{-2}) was obtained *via* a higher number of marketable fruits (19.1 fruits m^{-2}). When compared landraces, L1 was the most productive (9.4 kg m^{-2}) and L4 the least productive (5.0 kg m^{-2}) together with the highest waste production (18.2%). Landrace 2 had a good level of total production (7.9 kg m^{-2}) together with the lowest waste (7.9%) and did not significantly differ in terms of marketable production from L1 landrace. The number of marketable fruits per m^2 differed significantly among landraces, with the greatest number obtained for L4 (30.3) and the lowest for L3 (10.4); no significant differences were found between landraces 2 and 3 in terms of number of marketable fruits. Average marketable fruit weight was the highest for the round-shaped-fruits landraces L1 and L3 (676 and 624 g, respectively) and the lowest for the cylindrical L4 (166 g). No significant interaction was found between landraces (L) and grafting/ungrafting technique (T) in terms of fruit yields and characteristics.

The results of high performance liquid chromatography (HPLC) analysis of major polyphenols in fruit peel extracts for the four landraces grafted and ungrafted are presented in Table 2. Individual phenolic phytochemicals were grouped in: phenylamides (PhA), chlorogenic acid derivates (Cad) and other esters of quinic acids (Oeq). We found the following PhA: N-caffeoylputrescine, N-caffeoylputrescine derivatives and hydroxycinnamoyl amide.

Regardless of propagation technique, total PhA were significantly higher in L2 (9.89 μg mL^{-1}) than in L4 (4.40 μg mL^{-1}) and L3 (3.67 μg mL^{-1}) which in turn was significantly higher than in L1 (1.35 μg mL^{-1}). Total PhA content in

Table 1 Yield production of four landraces of eggplant grafted and ungrafted

		Total production (kg m^{-2})	Marketable production (kg m^{-2})	Number of marketable fruits m^{-2}	Weight marketable fruit (g)	Waste production (%)
Treatment	Ungrafted	6.7 b	5.8 b	16.1 b	502.6 NS	14.3 NS
	Grafted	7.7 a	6.6 a	19.1 a	487.5 NS	14.6 NS
Landrace[1)]	L1	9.4 a	8.0 a	14.1 bc	676.0 a	15.2 c
	L2	7.9 b	7.3 a	15.5 b	515.0 b	7.9 d
	L3	6.4 c	5.4 b	10.4 c	623.7 a	16.5 b
	L4	5.0 d	4.1 c	30.3 a	165.5 c	18.2 a
Interaction		NS	NS	NS	NS	NS

[1)] Landrace Bianca L1, Sciacca L2, Marsala L3 and Sicilia L4. The same as below.
In each column and for each fixed factor, values followed by same letters are not statistically different according to Duncan test ($P \leq 0.05$). Interactions are not significant (NS) at $P=0.05$.

Table 3 High performance liquid chromatography (HPLC) polyphenolic fingerprint and values in of major polyphenols in fruit peel extracts of grafted and ungrafted eggplant landraces (Bianca L1, Sciacca L2, Marsala L3 and Sicilia L4) (μg mL^{-1})

	Significance			L				T[1)]		L×T							
	L	T	L×T	L1	L2	L3	L4	Ungrafted	Grafted	L1× Ungrafted	L1× Grafted	L2× Ungrafted	L2× Grafted	L3× Ungrafted	L3× Grafted	L4× Ungrafted	L4× Grafted
N-Caffeoylputrescine	***	***	***	0.50 d	1.55 b	1.37 c	1.79 a	1.23	1.37	0.27 d	0.72 c	0.86 c	2.23 a	1.94 ab	0.79 c	1.84 b	1.74 b
N-Caffeoylputrescine derivatives	***	*	***	0.42 c	2.85 a	1.32 b	1.44 b	1.33	1.68	0.25 d	0.64 d	1.78 bc	3.91 a	2.03 b	0.61 d	1.31 c	1.56 c
Hydroxycinnamoyl amide	***	***	***	0.44 c	5.50 a	0.99 bc	1.37 b	1.18	2.96	0.28 c	0.59 c	1.45 b	9.55 a	1.58 b	0.39 c	1.42 b	1.32 b
Total phenylamides	***	***	***	1.35 c	9.89 a	3.67 b	4.60 b	3.74	6.01	0.75 e	1.95 d	4.09 cd	15.69 a	5.55 b	1.79 d	4.57 c	4.62 c
Caffeoylquinic acid	***	***	***	2.14 d	16.26 a	13.27 b	6.74 c	10.1	9.10	0.72 e	3.55 f	12.85 c	19.66 b	22.07 a	4.47 e	4.75 e	8.72 d
5-Caffeoylquinic acid	***	***	***	0.00 c	0.70 b	1.47 a	0.66 b	0.85	0.56	0.00 d	0.00 d	0.15 c	1.25 b	2.89 a	0.05 c	0.36 c	0.95 bc
3-5-Dicaffeoylquinic acid	***	*	***	0.00 d	1.48 a	1.23 b	0.46 c	0.89	0.70	0.00 e	0.00 e	0.78 c	2.18 b	2.44 a	0.02 d	0.33 c	0.59 c
4-5-Dicaffeoylquinic acid	***	NS	***	0.00 c	0.44 b	0.84 a	0.48 b	0.50	0.38	0.00 e	0.00 e	0.00 e	0.87 b	1.65 a	0.02 d	0.34 c	0.62 bc
1-5-Dicaffeoylquinic acid	***	***	***	0.00 c	0.43 b	3.13 a	0.34 b	1.56	0.38	0.00 d	0.00 d	0.00 d	0.85 b	6.24 a	0.01 c	0.01 c	0.66 b
Total chlorogenic acid derivates	***	***	***	2.14 c	19.30 a	19.93 a	8.67 b	13.9	11.12	0.72 f	3.55 ef	13.78 c	24.81 b	35.29 a	4.57 e	5.79 e	11.54 d
Feruloylquinic acid	***	NS	***	0.09 d	1.36 b	2.15 a	0.87 c	1.10	1.13	0.06 e	0.13 e	0.55 de	2.12 b	3.49 a	0.81 d	0.30 e	1.43 c
3-5-Caffeoylferuloylquinic acid	***	***	***	0.01 c	0.23 b	0.32 b	0.61 a	0.21	0.37	0.00 e	0.03 d	0.09 d	0.36 c	0.61 b	0.03 d	0.15 cd	1.06 a
4-5-Caffeoylferuloylquinic acid	***	***	***	0.01 c	1.54 b	9.57 a	0.50 c	5.21	0.60	0.02 de	0.00 e	1.69 b	1.38 bc	18.96 a	0.18 d	0.16 d	0.84 c
Caffeoylsinapylquinic acid	***	NS	***	0.64 bc	1.49 a	1.07 ab	0.14 c	0.77	0.90	1.07 c	0.20 d	0.04 e	2.93 a	1.95 b	0.19 d	0.00 f	0.27 d
Total others ester of quinic acids	***	***	***	0.76 d	4.58 b	13.11 a	2.11 c	7.29	2.99	1.15 d	0.36 d	2.37 c	6.79 b	25.01 a	1.21 d	0.61 d	3.60 c
Total phenolics	***	***	***	4.24 c	33.74 a	36.71 a	15.37 b	24.92	20.12	2.67 f	5.86 e	20.24 c	47.29 b	65.85 a	7.57 de	10.97 d	19.76 c

[1)]T, grafting/ungrafting technique.
Different letters in rows denote significant differences in treatment (Duncan test, $P<0.05$). The significance is designated by asterisks as follows: *, statistically significant differences at P-value below 0.05; **, statistically significant differences at P-value below 0.01; ***, statistically significant differences at P-value below 0.001. NS=not significant.

grafted plants (6.01 μg mL^{-1}), averaged over landraces, was higher than in ungrafted plants (3.74 μg mL^{-1}). ANOVA showed a significant interaction of landraces×grafting/ungrafting technique (L×T). The highest PhA content was recorded in fruits obtained from grafted L2 plants (15.69 μg mL^{-1}), whereas the lowest content was found in L1 ungrafted plants (0.75 μg mL^{-1}).

In the fruit peel we found the following Cad: caffeoylquinic acid, 5-caffeoylquinic acid, 3-5-dicaffeoylquinic acid, 4-5-dicaffeoylquinic acid, and 1-5-dicaffeoylquinic acid. Landraces 3 and 2, regardless of propagation technique, showed significantly higher total Cad content (19.93 and 19.30 μg mL^{-1}, respectively) than L4 (8.67 μg mL^{-1}) which in turn gave higher content than L1 (2.14 μg mL^{-1}). Chlorogenic acid derivates averaged over landraces accounted for 13.9 and 11.2 μg mL^{-1}, respectively in ungrafted and grafted plants. ANOVA for Cad showed a significant effect of the interaction L×T. With the exception of the combination L3×ungrafted plants, total Cad fruit content was significantly higher in L2, L4, and L1 grafted plants (24.81, 11.54, and 3.55 μg mL^{-1}, respectively) than in L2, L4, and L1 ungrafted plants (13.78, 5.79 and 0.72 μg mL^{-1}, respectively). Among Cad, caffeoylquinic acid was the most represented for all four landraces. Data collected on this compound supported the trend established for Cad. Landrace 2 plants, regardless of propagation technique, gave the highest caffeoylquinic acid fruit content (16.26 μg mL^{-1}), followed by L3 (13.27 μg mL^{-1}) which in turn showed higher content than L4 and L1 plants. ANOVA for caffeoylquinic acid showed a significant effect of the interaction L×T; fruit peel of ungrafted L3 plants had the highest caffeoylquinic acid content (22.07 μg mL^{-1}), followed by those harvested from grafted L2 plants (19.66 μg mL^{-1}) which in turn showed a higher content than grafted L4 plants (8.72 μg mL^{-1}). There was no significant difference in caffeoylquinic acid fruit content between grafted L3 (4.47 μg mL^{-1}) and ungrafted L4 plants (4.75 μg mL^{-1}); fruits from L1 grafted plants had a significantly lower content than fruits from L2, L3 and L4 either grafted or ungrafted, but significantly higher (3.55 μg mL^{-1}) than fruits from L1 ungrafted plants (0.72 μg mL^{-1}).

We found the following Oeq: feruloylquinic acid, 3-5-caffeoylferuloylquinic acid, 4-5-caffeoylferuloylquinic acid and caffeoylsinapylquinic acid. Among these compounds, 4-5-caffeoylferuloylquinic acid was the most represented. Fruit peel from ungrafted L3 plants had the highest 4-5-caffeoylferuloylquinic acid content (18.96 μg mL^{-1}), followed by those from

grafted L2 plants (1.38 μg mL^{-1}).

Regardless of propagation technique, L2 and L3 landraces showed a significantly higher total polyphenol content (33.74 and 36.71 μg mL^{-1}, respectively) than L4 (15.37 μg mL^{-1}) which in turn showed a significantly higher content than L1 (4.24 μg mL^{-1}). Fruits from ungrafted plants, regardless of landraces tested, were superior to grafted plants in terms of total polyphenol content. However, a significant interaction was found between landrace and propagation technique; the highest total polyphenol content was identified in fruits from ungrafted L3 plants (65.85 μg mL^{-1}) and in those from grafted L2 plants (47.29 μg mL^{-1}). The lowest total polyphenol content was detected in L1 plants, whatever grafted (5.86 μg mL^{-1}) or ungrafted (2.67 μg mL^{-1}).

3. Discussion

In our experiment, grafting has proved a useful technique to increase the eggplant productive potential of four eggplant landraces. Although we did not find any significant interaction between landraces and grafting for yield production, grafted plants gave both a significantly higher total and marketable yield production, and a higher number of marketable fruits than ungrafted ones, without negatively affecting average fruit weight and waste production. Our results are in accord with those obtained by Maršič *et al.* (2014) who, by investigating the yield response of three commercial varieties and one landrace of eggplant, found that grafted plants produced consistently more fruits per plant than ungrafted ones. Higher yield in grafted vegetables has been attributed to increased absorption of water and nutrients (Lee 1994; Colla *et al.* 2006). The significant differences found in the four landraces analyzed, concerning the average weight of the marketable fruits, are probably due to the berry shape typology, elongated fruits, such as those of L2 and L4 landraces, being generally lighter than round or globose fruits, such as those of L1 and L3 landraces.

In the present study, in general, grafting increased total PhA content. Phenylamides are low-molecular products of covalent bonding between carboxylic groups of hydrocynnamic acids and amine groups of aliphatic di- and polyamines or aromatic monoamines (Edreva *et al.* 2007). The multiplicity of their chemical properties, bonding types, interactions and functions have suggested their involvement in important processes in plants including growth, development and stress defense, therefore suggesting their possibility inclusion to a new class of growth regulators (Edreva *et al.* 2007). In this respect, the grafting beneficial effects on yield production observed in our study could be attributed not only to the well-known grafting advantages (improved water and nutrient uptake, enhanced vigor, and tolerance to biotic and abiotic stress) but also to an increase in PhA content. Improved growth and development in tomato grafted plants were related by Sánchez-Rodríguez *et al.* (2011) to N-caffeoylputrescine, N-caffeoylputrescine derivatives, and hydroxycinnamoyl amide.

In the present study, five caffeoylquinic acid derivates were identified. All the isolated compounds are reported having antioxidant activity and being effective in free radical scavengers (Stommel and Whitaker 2003; Hung *et al.* 2006; Luthria and Mukhopadhyay 2006; Singh *et al.* 2009; Luthria *et al.* 2010).

Our study also showed that grafting increased total polyphenol fruit content in three out of four landraces (Table 2). This findings are different from those of Moncada *et al.* (2013), who reported little or no effect of grafting on phenolic content in fruits of eggplant commercial varieties, but are consistent with those of Maršič *et al.* (2014) who reported that grafting significantly increased phenolic concentration in fruits from a grafted eggplant landrace as opposed to commercial varieties. Our results also partially agree with those of Gisbert *et al.* (2011) who found a higher phenolic content in the eggplant Black Beauty grafted on *Solanum macrocarpon* as opposed to ungrafted plants. The mechanism of action leading to an accumulation in phenolic compounds has been generally associated to a plant stress condition (Dixon 1995; Moglia 2008), such as that determined by certain scion/rootstock combinations (Maršič *et al.* 2014). Our results might confirm this theory. On the other hand, in the present study, the lower total polyphenol content of grafted L3 plants indicates that this landrace/rootstock combination substantially reduced the phenolic metabolism of the grafted plants as compared to the ungrafted ones. This different response to grafting could be related to the lower number of fruits produced from L3 ungrafted plants which might have induced an earlier physiological fruit maturity and consequently a higher phenolic content as compared to the grafted plants. On this respect, our result would be consistent with the assumption of Lee *et al.* (2010) that the benefit of grafting depends on the scion-rootstock combination and would be also in accord with Maršič *et al.* (2014) who, by testing an eggplant landrace grafted onto tomato rootstock Beaufort F1, suggested a similar hypothesis.

It is well known that new cultivars of eggplant provide increasingly high production. However, breeders did not take so far into account the secondary metabolites present in the fruits. On the other hand, research efforts to produce fruits and vegetables rich in compounds important for human health are steadily increasing (Scheerens 2001; Rouphael *et al.* 2010). Therefore, we focused our attention on local populations which, although not comparable with modern F1 hybrids as regards 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 and particularly if they present a high content of phytochemicals having beneficial effects on human health. The results presented in this paper are noteworthy principally for demonstrating that among the four landraces tested, landrace 2, characterized by black epidermal tissue and pyriform fruit shape, when grafted onto *S. torvum* not only gave higher marketable yield and number of fruits than L2 ungrafted plants, but also showed a higher phenolic antioxidant fruit content. Consequently this landrace/rootstock combination should deserve more attention by plant nurseries involved in grafted seedling production. We also demonstrated that landrace 4, characterized by black epidermal tissue and small cylindrical fruits would benefit, if grafted onto *S. torvum*, from a substantial increase in antioxidant fruit characteristics. As the consumers' demand for low weight, small eggplant fruits is consistently increasing, enhancing fruit quality of L4 would result in an increase of its market potentiality.

4. Conclusion

In conclusion, in those areas of Sicily (Southern Italy), where vegetable crops are still carried out mostly by traditional methods and modern cultivation methods are adopted slowly, the grafting technique proposed in this paper could help in increasing yield and fruit quality characters and consequently in providing higher profits to farmers who grow determinate eggplant landraces. Our results would become more promising and useful if medical studies would confirm the major role of eggplant polyphenols in preventing several human diseases.

5. Materials and methods

5.1. Plant material and cultivation

The study was carried out at the experimental fields of the Department of Agricultural and Forest Sciences of Palermo (longitude 13°19′E, latitude 38°09′N) in the northern coast of Sicily (Italy). Four grafted and ungrafted eggplant landraces cultivated in Sicily: Bianca (L1) with white epidermal tissue and ovoid fruit shape, Sciacca (L2) with black epidermal tissue and pyriform fruit shape, Marsala (L3) with purple epidermal tissue and globular fruit shape), and Sicilia (L4) with black epidermal tissue and cylindrical fruit shape (Fig. 1) were compared. *Solanum torvum* Sw. seedlings were used as rootstock. For the production of grafted plant material, rootstock seeds (Agriseeds srl, Viadana, Mantova, Italy) were planted in 44-cell seedling trays, under a temperature regime of 25°C/18°C (day/night) in a propagation greenhouse. After 20 days, seeds from the 4 landraces were

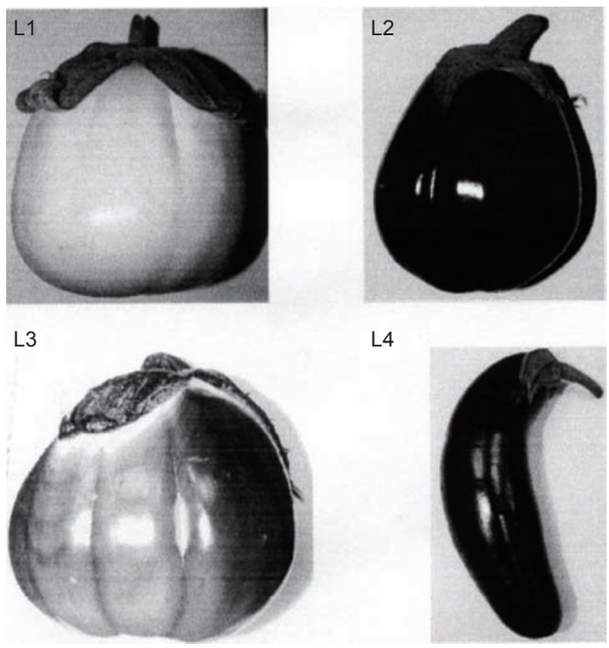

Fig. 1 Landrace Bianca L1, Sciacca L2, Marsala L3 and Sicilia L4.

planted in 104-cell trays and given the same temperature regime and planting method as the rootstock. Trays were watered manually every day to maintain the substrate at field capacity. Seventy-five days after planting all seedlings had reached an adequate diameter to allow for grafting. The grafting was carried out using the tube grafting method as described by Lee *et al.* (2010). The grafting involved cutting off the rootstock at a 45° angle and making a similar cut on the scion. Attention was paid to be sure that the diameters of the rootstock/scion were nearly identical so that the two exchange sites fitted perfectly. The grafting technique used was completed by attaching a silicon tube in the grafting point to ensure the correct fit and the correct amount of pressure was applied. The grafted plants were misted and maintained at a temperature of 20°C and a humidity rate of 95% for 7 days to encourage histological processes. Healing was completed 7 days after 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.

The field trial was conducted in a Typic Rhodoxeralf soil. The soil was prepared by making a medium-deep plowing (35 cm) and a reduction of the earth aggregates achieved by mechanical rotating means. Pre-planting fertilization was carried out over the whole of the soil surface using 66 kg nitrogen ha^{-1}, 132 kg phosphorous pentoxide ha^{-1} and 96 kg potassium oxide ha^{-1}. Plug plants were transplanted into the open field on May 10th, 2013 in the centre of black polyethylene (PE) film (thickness of 20 μm) mulched plots

on single rows 100 cm apart. In row spacing was 0.50 cm. A completely randomized block design was used, with four eggplant landraces and two propagation techniques (grafting and ungrafting). Three replicates for each combination and ten plants per replication were used. During the growing period the crop received, by a drip irrigation system 250 kg nitrogen ha^{-1}, 150 kg phosphorous pentoxide ha^{-1} and 250 kg potassium oxide ha^{-1}.

5.2. Weather conditions

Average daily temperature during the experimental period from May to August 2013 was obtained from the meteorological station of the experimental farm of the Department of Agricultural and Forest Sciences, University of Palermo, Italy (Table 3). In terms of temperatures, the weather during the experimental period in 2013 was comparable to the long-term average even though the average daily temperatures were slightly below the long term average, with the highest negative deviation in June (by 1.7°C).

5.3. Harvests and fruit sampling

Total production (kg m^{-2}), marketable production (kg m^{-2}), number of marketable fruits per m^2, average weight of marketable fruits (g) and waste production (%) were recorded.

Sampling for the quality analysis of the fruits was carried out using 3–5 commercially mature fruits for each replication from the second and third harvest; 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 fruits.

5.4. Samples preparation for chemical analysis

Immediately after receipt, fresh fruit samples were washed thoroughly with cold tap water to remove adhering extraneous matter. After that, they were peeled and the skin was cut into small pieces using stainless steel knife. Fruit skin samples were spread on netted trays and were dried in hot air oven at 60°C until the equilibrium moisture levels were attained. A heating/drying oven with natural convection (Model Binder GmbH ED 115 Instruments, Tuttlingen) was used. Dried skin eggplant pieces were mashed, passed through a standard 20 mesh size, and packed in Pet jars (Medfor, Aldershot, Hampshire, UK) until extracted and analyzed.

5.5. Polyphenol extraction and analysis

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, volume/weight (v/w), extraction temperature from 10 to 60°C, extraction solvent concentration from 0.5 to 2%, extraction time from 0 to 1 h. 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°C. The operation was repeated three times on the same samples 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 (40°C and 7 200 N m^{-2}, respectively). 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 high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) analysis.

5.6. Instrument conditions

Phenolic compounds were identified using high performance liquid chromatography coupled with tandem mass spectrometry (HPLC/MS/MS). The HPLC system (LC-MS-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 microliters of the extract were 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^{-1}, the following linear

Table 3 Monthly meteorological data from May to August of 2013 from the meteorological station of the experimental farm of the Department of Agricultural and Forest Sciences, University of Palermo, Italy (°C)

Month	Monthly air temperature	Temperature deviation from the 1986−2015 average	Maximum air temperature for the month	Minimum air temperature for the month
May	18.9	–0.6	22.0	16.3
June	21.4	–1.7	23.7	18.8
July	21.4	–0.6	28.1	22.3
August	26.8	–0.1	29.5	23.8

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 tandem 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 1 200 m/z. Data acquisition and processing were performed using a Mass-Lynx NT 3.5 data system (Micromass Inc., Milan, Italy).

5.7. 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-Aldrich) to quantify feruloylquinic acid, 3,5-caffeoylferuloylquinic acid, 4,5-caffeoylferuloylquinic acid, caffeoylsinapylquinic acid at the concentration from 0.05 to 250 μg mL^{-1}. 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 (SD) of the blank signal as follows:

$y_D = y_b + 2t\mathrm{SD}$

$y_Q = y_b + 10\mathrm{SD}$

Where, t is a constant of the *t*-Student distribution (one-tailed) dependent on the confidence level and degrees of freedom (d*f*). A 95% confidence level was chosen. For y_b and SD determination, 9 blank measurements were performed by injection of 5 μL of blank. Limit of 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).

5.8. Experimental design and statistical analysis

Treatments were defined by a two-factorial experiment in three randomized replications. The first factor had four levels (four landraces), whereas the second factor had two levels (grafting and ungrafting). Percentage data were subjected to arcsin transformation before ANOVA analysis. Mean separation was performed by Duncan multiple range test ($P \leq 0.01$). The means for landraces (L), grafting/ungrafting technique (T) and the interaction landraces×grafting/ungrafting technique (L×T) from the ANOVA table are presented. All the statistical analysis were performed using SPSS software version 14.0 (StatSoft, Inc., Chicago, USA).

References

Bletsos F, Thanassolopoulos C, Roupakias D. 2003. Effect of grafting on growth, yield and *Verticillium wilt* of eggplant. *HortScience*, **38**, 183–186.

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

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. *HortScience*, **41**, 622–627.

Concellon A, Anon M C, Chaves A R. 2004. Characterization and changes in polyphenol oxidase from eggplant fruit (*Solanum melongena* L.) during storage at low temperature. *Food Chemistry*, **88**, 17–24.

D'Anna F, Sabatino L. 2013. Morphological and agronomical characterisation of eggplant genetic resources from the Sicily area. *Journal of Food, Agriculture & Environment*, **11**, 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, Fabaceae, Liliaceae, Solanaceae, and Umbelliferae*. Springer, New York, USA. pp. 163–220.

Dixon R A, Paiva N. 1995. Stress-Induced phenylpropanoid metabolism. *The Plant Cell*, **7**, 1085–1097.

Edreva A M, Velikova, V B, Tsonev T D. 2007. Phenylamides in plants. *Russian Journal of Plant Physiology*, **54**, 325–341.

Gisbert C, Prohens J, Nuez F. 2011. Performance of eggplant grafted onto cultivated, wild, and hybrid materials of eggplant and tomato. *International Journal of Plant Production*, **5**, 367–380.

Hanson P M, Yanga R, Tsoua S C S, Ledesmaa D, Englea L, Leeb T. 2006. Diversity in eggplant (*Solanum melongena*) for superoxide scavenging activity, total phenolics, and ascorbic acid. *Journal of Food Composition and Analysis*, **19**, 594–600.

Huang H Y, Chang C K, Tso T K, Huang J J, Chang W W, Tsai W C. 2004. Antioxidant activities of various fruits and vegetables produced in Taiwan. *International Journal of Food Science and Nutrition*, **55**, 423–429.

Hung T M, Na M, Thuong P T, Su N D, Sok D, Song K S, Seong Y K, Bae K. 2006. Antioxidant activity of caffeoyl quinic acid derivatives from the roots of *Dipsacum asper* Wall. *Journal of Ethnopharmacololgy*, **108**, 188–192.

Kaur C, Kapoor H C. 2001. Antioxidants in fruits and vegetables — The millennium's health. *International Journal of Food Science and Technology*, 36, 703–725.

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

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

Lee J M, Kubota C, Tsao S J, Bie Z, Hoyos Echevarria P, Morra L, Oda A M. 2010. Current status of vegetable grafting: diffusion, grafting techniques, automation. *Scientia Horticulturae*, **127**, 93–105.

Luthria D L, Mukhopadhyay S. 2006. Influence of sample preparation on assay of phenolic acids from eggplant. *Journal of Agricultural and Food Chemistry*, **54**, 41–47.

Luthria D, Singh A P, Wilson T, Vorsa N, Banuelos G S, Vinyard B T. 2010. Influence of conventional and organic agricultural practices on the phenolic content in eggplant pulp: Plant-to-plant variation. *Food Chemistry*, **121**, 406–411.

Maršič N K, Mikulič-Petkovšek M, Štampar F. 2014. Grafting influences phenolic profile and carpometric traits of fruits of greenhouse-grown eggplant (*Solanum melongena* L.). *Journal of Agricultural and Food Chemistry*, **62**, 10504–10514.

Moglia A, Lanteri S, Comino C, Acquadro A, De Vos R, Beekwilder J. 2008. Stress-induced biosynthesis of dicaffeoylquinic acids in globe artichoke. *Journal of Agricultural and Food Chemistry*, **56**, 8641–8649.

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.). *Scientia Horticulturae*, **149**, 108–114.

Raigon M D, Prohens J, Munoz-Falcon J E, Nuez F. 2008. Comparisons of eggplant landraces and commercial varieties for fruit content of phenolics, minerals, dry matter and protein. *Journal of Food Composition and Analysis*, **21**, 370–376.

Raigon M D, Rodriguez-Burruezo A, Prohens J. 2010. Effects of organic and conventional cultivation methods on composition of eggplant fruits. *Journal of Agricultural and Food Chemistry*, **58**, 6833–6840.

Raimondo F M, Gianguzzi L, Ilardi V. 1992. Inventario delle specie "a rischio" nella flora vascolare della Sicilia (An inventory of the endangered vascular Sicilian flora). *Quaderni di Botanica Ambientale Applicata,* **3**, 65–132. (in Italian)

Rouphael Y, Schwarz D, Krumbein A, Colla G, 2010. Impact of grafting on product quality of fruit vegetables. *Scientia Horticulturae*, **127**, 172–179.

Sánchez-Rodriguez E, Ruiz J M, Ferreres F, Moreno D A. 2011. Phenolic metabolism in grafted versus nongrafted cherry tomatoes under the influence of water stress. *Journal of Agriculture and Food Chemistry*, **59**, 8839–8846.

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

Scheerens J C. 2001. Phytochemicals and the consumer: factors affecting fruit and vegetable consumption and the potential for increasing small fruits in the diet. *HortTechnology*, **11**, 547–556.

Schiavi M, Masera R, Lorenzoni C, Soressi G P. 1991. Metodi utilizzati per il miglioramento genetico della Dorata di Parma (Breeding methods for the improvement of "Dorata di Parma"). *Agricoltura e Ricerca*, **82**, 21–30. (in Italian)

Schippmann U, Leaman D J, Cunningham A B. 2002. Impact of cultivation and gathering of medicinal plants on biodiversity: Global trends and issues. In: *Biodiversity and the Ecosystem Approach in Agriculture*. Foresty and Fisheries, FAO, Rome. P. 21.

Singh P K, Gopalakrishnan T R. 1997. Grafting for wilt resistance and productivity in brinjal (*Solanum melongena* L.). *Horticultural Journal*, **10**, 57–64.

Singh A P, Luthria D, Wilson T, Vorsa N, Singh V, Banuelos G S, Pasakdee S. 2009. Polyphenols content and antioxidant capacity of eggplant pulp. *Food Chemistry*, **114**, 955–961.

Steinmetz K A, Potter J D. 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 content and composition of eggplant fruit in a germplasm core subset. *Journal of the American Society for Horticultural Science*, **128**, 704–710.

Todaro A, Comino F, Rapisarda P, Catalano A, Barbagallo R N, Spagna G. 2009. Recovery of anthocyanins from eggplant peel. *Food Chemistry*, **114**, 434–439.

(Managing editor WENG Ling-yun)