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Review

Amelioration of heavy metal and nutrient stress in fruit vegetables by grafting

Dimitrios Savvas[a,*], Giuseppe Colla[b], Youssef Rouphael[c], Dietmar Schwarz[d]

[a] Agricultural University of Athens, Department of Crop Science, Laboratory of Vegetable Crops, Iera Odos 75, 11855 Athens, Greece
[b] Dipartimento di Geologia e Ingegneria Meccanica, Naturalistica e Idraulica per il Territorio, Università della Tuscia, 01100 Viterbo, Italy
[c] Department of Crop Production, Faculty of Agricultural Engineering and Veterinary Medicine, Lebanese University, Dekwaneh-El Maten, Lebanon
[d] Institute of Vegetable and Ornamental Crops, Grossbeeren&Erfurt e.V., Theoder Echtermeyer Weg 1, D-14979 Großbeeren, Germany

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ABSTRACT

The response of grafted vegetables to stress conditions owing to the nutrient status, and the presence of heavy metals in the root environment may be different than that of self-rooted plants, depending mainly on the rootstock genotype. Several studies have indicated that some rootstocks are capable of restricting the uptake and/or the transport of heavy metals (e.g. Cd, Ni, Cr) and micronutrients (e.g. Cu, B and Mn) to the shoot, thereby mitigating the stress caused by excessive external concentrations of them. However, other mechanisms driven by the root system, such as detoxification of harmful elements or hormonal signals modifying gene expression in the scion, seem to be involved in the mitigation of stress caused by excessive external nutrient or heavy metal concentrations. On the other hand, the uptake and/or utilization efficiency of macronutrients (N, P, K, Ca and Mg) by plants may be enhanced by grafting onto some rootstocks. This is ascribed mainly to the root characteristics of these rootstocks, which are more vigorous than those of highly productive cultivated varieties. However, other mechanisms implicated in the efficiency of active nutrient absorption by the roots, as well as signals arising from the scion, which are mainly governed by sink demand, may also enhance nutrient uptake and utilization. The higher efficiency of some graft combinations of fruit vegetables to take up and utilize nutrients may mitigate yield losses owing to shortages of these nutrients in the root environment of plants and restrict nutrient losses due to leaching. Nevertheless, it is important to specifically test each grafting combination and not merely each rootstock for its ability to ameliorate nutrient or heavy metal stress because in many instances the responses depend on the rootstock/scion combination. This report gives an overview on the prospects and restrictions of grafting as a means to minimize the negative effects of heavy metals, excessive nutrient availability, nutrient deficiency, and alkalinity stress on vegetable crop performance taking into consideration agronomical, physiological and biochemical aspects.

Contents

1. Introduction

Due to the limited availability of arable land, the high demand for off-season vegetables and the intensive farming practices with limited crop rotations, vegetables are often cultivated under unfavourable conditions which induce stress. These conditions include environments that are too cold, wet or dry, hypoxia, salinity, heavy metal contaminations, excessive and insufficient nutrient availability, and soil pH stress. These conditions cause various physiological and pathological disorders leading to severe crop loss. One environmentally-friendly technique for avoiding or reducing losses in production caused by abiotic stress conditions in high-yielding genotypes belonging to *Solanaceae* and *Cucurbitaceae* families would be to graft them onto rootstocks capable of reduc-

* Corresponding author. Tel.: +30 210 5294510; fax: +30 210 5294504.
E-mail address: dsavvas@aua.gr (D. Savvas).

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ing the negative effect of external stress on the shoot. Grafting is the union of two or more pieces of living plant tissue, which are forced to develop vascular connection and grow as a single plant. In the past, grafting was used widely in fruiting vegetable crops to limit the effects of soil pathogens (Lee, 1994; Crinò et al., 2007; Bletsos and Olympios, 2008), but the reasons for grafting as well as the vegetable species and cultivars grafted have increased dramatically over the years. Several contributions included in the present issue focus on specific advantages of grafting and their possible applications in commercial vegetable production.

One of the possible applications of grafting in commercial vegetable production is the mitigation of stress caused by adverse chemical soil conditions in the root environment. Plants exert genetic control on the uptake of nutrients and non-nutrient elements through the root structure, the uptake mechanisms in the root cell at biochemical level, and the signals arising from the shoot, which are mainly governed by sink demand (Marschner, 1995). Since both the root structure and the uptake efficiency of the root cells are determined by the rootstock, it is reasonable to assume that plants grafted onto different rootstocks may exhibit dissimilar abilities to take up nutrients and other non-nutrient elements. Indeed, an appreciable body of related investigations has indicated that grafting may limit nutrient and heavy metal toxicity (Edelstein et al., 2005; Rouphael et al., 2008b; Arao et al., 2008; Savvas et al., 2009), or enhance nutrient uptake efficiency (Ruiz et al., 1997; Colla et al., 2010a; Rouphael et al., 2008a) and alkalinity tolerance (Colla et al., 2010b). Hence, grafting fruit vegetables onto appropriate rootstocks may be used to mitigate or even eliminate yield restrictions owing to nutrient and heavy metal toxicities and minimize intake of heavy metals by consumers. Furthermore, grafting may be used to increase fertilizer use efficiency and prevent nutrient deficiencies in marginally fertile soils.

The aim of this paper is to review the recent literature on the responses of grafted plants to adverse chemical soil conditions, in particular insufficient or toxic levels of individual nutrients, excessive concentrations of heavy metals, and too low or too high pH levels. The agronomical, physiological and biochemical processes conferring tolerance to adverse chemical soil conditions in grafted plants will be also covered. The review will conclude by identifying several prospects for future researches aiming to improve the role of grafting in vegetable crops grown under adverse chemical soil conditions.

2. Heavy metal tolerance

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). Non-nutrient heavy metals such as cadmium, arsenic, lead, and mercury, which are harmful for both plants and humans, are introduced to agricultural ecosystems from various sources, including industry, reclaimed wastewater, and soil amendments originating from various sources (Diacono and Montemurro, 2010; Gupta et al., 2010). Although the problem of heavy metal contamination in fruit vegetables is currently not widespread, some recent reports are worrying. Thus, as indicated in a survey conducted in Japan (Arao et al., 2008), approximately 7% of eggplant (*Solanum melongena* L.) fruit contain cadmium at concentrations exceeding the internationally acceptable limit for fruiting vegetables. In addition to its adverse impact on human health, Cd has been reported to seriously affect important plant processes, including water transport, nitrogen metabolism, oxidative phosphorylation in mitochondria, photosynthesis and chlorophyll content (Burzynski and Klobus, 2004; Djebali et al., 2005; López-Millán et al., 2009; Feng et al., 2010). The impact of Cd on plant growth and development depends not only on its concentration in the external medium, but also on the plant genotype, plant part and duration of exposure (Clemens, 2001; Moustakas et al., 2001; Zhang and Shu, 2006; DalCorso et al., 2008). Recent research has indicated that some rootstocks of fruit vegetables may restrict the uptake of heavy metals (Arao et al., 2008; Rouphael et al., 2008a; Mori et al., 2009). Hence, grafting fruit vegetables onto appropriate rootstocks, may limit the heavy metals accumulation in the aerial parts, thereby mitigating their adverse effects on crop performance and human health.

Fruit vegetables, such as tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), and eggplant (*S. melongena* L.), 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 has been so far hardly investigated. One of the few reports is that of Arao et al. (2008) who were able to reduce Cd concentrations in eggplant fruit by grafting onto *Solanum torvum*. In particular, grafting *S. melongena* plants onto *S. torvum* reduced the leaf and stem Cd concentrations by 67–73% in comparison to self-grafting or grafting onto *Solanum integrifolium*, in both Cd-polluted and unpolluted soils. The Cd concentration in xylem sap collected from stems of *S. torvum* was 22% of that in stems of *S. melongena*, indicating an appreciable restriction of the Cd translocation from root to shoot in the former. However, the concentrations of Cd in the roots of *S. melongena* and *S. torvum* were similar when the plants were exposed to identical external Cd levels (Mori et al., 2009). These results indicate that *S. torvum* restricts specifically the translocation of Cd to the shoot and not the Cd uptake. Genotypic differences in the ability of the root to prevent Cd translocation to the shoot have been demonstrated also for soybean by means of grafting experiments (Sugiyama et al., 2007). Other results (Ntatsi and Savvas, unpublished) indicate that the rootstock may considerably influence the translocation of Ni and Pb to the shoot in grafted cucumber (*Cucumis sativus* L.). The mechanisms underlying the impediment of heavy metal uptake and translocation to the shoot by some rootstocks are still unclear. According to Mori et al. (2009), the restriction of Cd translocation to the fruit of eggplant grafted onto *S. torvum* in comparison to self-grafted *S. melongena* seems to be related to the process of xylem loading. 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). Moreover, Edelstein and Ben-Hur (2007) studied the effects of grafting on heavy metal and trace mineral concentrations in the fruit under field conditions, using melon plants (cv. 'Arava'), ungrafted and grafted onto the commercial Cucurbita rootstock 'TZ-148' and irrigated using marginal quality water. The concentrations of B, Zn, Sr, Mn, Cu, Ti, Cr, Ni, and Cd were lower in fruit from grafted 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. To our best knowledge, there are currently no other reports in the international literature concerning the impact of grafting on the uptake of non-nutrient heavy metals, such as Pb, Hg, or As by fruit vegetables.

3. Nutrient toxicity tolerance

Indiscriminate use of large amounts of chemicals, the use of reclaimed wastewater for irrigation, the application of sewage sludge or other contaminated soil amendments and other anthropogenic activities have dramatically increased the concentrations of several nutrient ions in soils used for open-field and protected cultivation (Guo et al., 2004; He et al., 2005; Yu et al., 2005). In these cases, soils may contain NO_3^-, SO_4^- $H_2PO_4^-$, K^+, Ca^{2+}, Mg^{2+} and metallic micronutrients (e.g. Cu) at excessively high levels, the impact of which is different than that of too high NaCl concentrations (Yu et al., 2005). Under high salt conditions, crop performance may be adversely affected by water deficit arising from the low water potential of the nutrient solution (osmotic effect). Furthermore, the crops can be damaged by salinity-induced nutritional disorders associated with excessive ion uptake or nutrient imbalance, and competitive uptake or partitioning within the plant (Grattan and Grieve, 1999; Munns and Tester, 2008).

Huang et al. (2010) showed that the crop performance of three grafting combinations of cucumber (*Cucumis staivus* L. cv. Jinchun No. 2), specifically self-grafted cucumber, or grafted onto the commercial rootstocks 'Black Seeded' figleaf gourd (*Cucurbita ficifolia* Bouché) and Chaofeng Kangshengwang (*Lagenaria siceraria* Standl.) respond differently to EC increases from 1.9 to 5.7 and 9.8 dS m^{-1} owing to elevated concentrations of macronutrients in the nutrient solution. Compared with the self-grafted plants, cucumber plants grafted onto 'Black Seeded' figleaf gourd had higher scion dry weight not only at 1.9 dS m^{-1} but also at 5.7 and 9.8 dS m^{-1}. These plants could significantly alleviate scion growth reduction, maintain higher soluble sugar and manganese (Mn) contents, higher superoxide dismutase (SOD) and peroxidase (POD) activities, but lower electrolyte leakage and malondialdehyde (MDA) at 5.7 dS m^{-1}. The authors concluded that grafting cucumber onto 'Black Seeded' figleaf gourd could increase plant tolerance to salinity induced by major nutrients.

Grafting cucumber, cv. 'Akito' onto the commercial rootstock 'Shintoza' (*Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne) restricted the uptake and translocation of Cu to the shoot, thereby mitigating the adverse effects of excessive Cu supply on plant biomass and fruit yield (Rouphael et al., 2008a). Thus, the leaf Cu concentration in grafted plants treated with a nutrient solution containing 47 and 94 μM Cu increased by 138% and 181%, respectively, in comparison with plants supplied with 0.3 μM Cu, while in ungrafted plants the increase in the leaf Cu level was 235% and 392%, respectively. Rouphael et al. (2008a) attributed the improved crop performance of grafted cucumber plants to the ability of the squash rootstock to restrict the accumulation of Cu in the shoot. These results indicate that Cu toxicity in cucumber cultivated in environments with too high Cu levels in the root zone may be partly mitigated by grafting onto the rootstock 'Shintoza'.

Savvas et al. (2009) found that the transport of Cu to the leaves of tomato 'Belladona' was also restricted when the plants were grafted onto the rootstock 'He-Man' (*Solanum lycopersicum* L. × *Solanum habrochaites* S. Knapp & D. M. Spooner). However, the concentration of Cu was significantly lower not only in the leaves but also in the roots of plants grafted onto 'He-Man' in comparison with self-grafted 'Beladona' plants. Moreover, the leaf Mg concentration was also restricted by grafting 'Belladona' onto 'He-Man' but this was accompanied by an increase in the root Mg level. These results indicate that the mechanisms involved in the restriction of the leaf Mg concentrations in plants grafted onto 'He-Man' are different than those reducing the leaf Cu levels. Nevertheless, the occurrence of direct Mg toxicity is rather unusual in tomato crops and thus the only nutrient toxicity stress that might be more efficiently tolerated by tomato when grafted onto 'He-Man' is that caused by excessive Cu in the root zone. Further research is needed to assess whether other commercial rootstocks are capable of mitigating Cu toxicity and elucidate the mechanisms underlying this response.

Stress due to boron toxicity may be a problem in many areas of the world (Reid, 2010), first because excessive B concentrations in the irrigation water and/or the soil are not unusual and second because the threshold between deficiency and toxicity is narrow (Mortvedt et al., 1991). The problems of excessive soil B is more frequent in dry areas of the world, such as the Mediterranean region and parts of Australia (Yau and Ryan, 2008). Boron toxicity can also be mitigated by grafting onto suitable rootstocks, as indicated by an experiment with melon (*Cucumis melo* L.) plants, which were exposed to five different B concentrations ranging from 0.1 to 10 mg L^{-1} in the irrigation water (Edelstein et al., 2005, 2007). In both experiments, the tissue B concentrations were significantly lower in melon plants grafted onto the commercial rootstock 'TZ-148' (*C. maxima* Duchesne × *C. moschata* Duchesne) than in self-rooted plants. The non-grafted plants were more sensitive to excess boron supply than the grafted ones in terms of fruit yield and dry weight accumulation in shoots and roots (Edelstein et al., 2005, 2007). These results suggest that grafting of fruit vegetables onto rootstocks, which are capable of restricting boron uptake, may alleviate or even prevent growth and yield decreases due to B toxicity.

The responses of grafted plants to stress caused by exposure to excessive external nutrient concentrations depends not only on the genotype of the rootstock but also on that of the scion. Although the rootstock genotype determines the uptake rate of a nutrient, the responses of the grafted plant to an excessive concentration of this nutrient is ultimately determined by the scion. Thus, a rootstock conferring tolerance to stress caused by excess external nutrient levels when used to graft a particular scion genotype, may prove to be completely inefficient in enhancing nutrient stress tolerance when used to graft other cultivars or species. This was demonstrated by Guimarães et al. (2009), who applied reciprocal grafting between a Zn hyperaccumulator, *Thlaspi caerulescens*, and a Zn nonaccumulator, *Thlaspi perfoliatum*. It was found that hyperaccumulation of Zn in leaves of the hyperaccumulator *T. caerulescens* was primarily dictated by root processes but the mechanisms controlling Zn hypertolerance in the hyperaccumulator *T. caerulescens* were driven primarily by processes localized in the shoot. These results emphasize the need to specifically test each grafting combination and not merely each rootstock for its ability to ameliorate stress owing to a nutrient toxicity. The search for suitable molecular markers and their use could be a very helpful tool to reduce the immense effort that would be necessary for such a comprehensive testing of grafting combinations.

4. Nutrient deficiency tolerance

Many rootstocks used to graft vegetables are wild genotypes of the same species as the scion, relatives, or hybrids of them, which are characterized by more vigorous root systems than those of highly productive cultivated varieties (Davis et al., 2008). In agreement with this consideration, Öztekin et al. (2009) found a significant increase in the root density by 25.3% on average in tomato plants grafted onto 'He-Man' and 'Beaufort', in comparison with self-grafted plants. The more vigorous root system of the rootstock results in increased nutrient and water uptake, and this may enhance the growth rate and yield performance of the whole plant (Lee, 1994). Indeed, many studies revealed that some graft combinations were significantly more efficient in absorbing and transporting nutrients to the shoot, such as phosphorus, nitrogen, potassium, magnesium, calcium, iron, or other micronutrients in comparison with non-grafted plants (Masuda and Gomi, 1984; Ruiz and Romero, 1999; Pulgar et al., 2000; Rivero et al., 2004; Rouphael et al., 2008b; Colla et al., 2010a,b; Salehi et al., 2010).

Grafting of *Cucurbitaceae* to some rootstocks seems to be especially beneficial for the nitrogen nutrition of these plants. Ruiz et al. (1997) grafted melon, cv. 'Yuma' and 'Gallicum', onto three *C. maxima* × *C. moschata* rootstocks, specifically 'Shintoza', 'RS-841' and 'Kamel', and reported that grafted plants were more efficient in taking up nitrogen. The foliar N concentrations correlated positively with the fruit yield. In a subsequent study, Ruiz and Romero (1999) found higher concentrations of NO_3^-, lower nitrate reductase activity (NRA), and greater contents of total free amino acids and soluble proteins in non-grafted melon plants in comparison with plants grafted onto *C. maxima* × *C. moschata* rootstocks. However, the tissue concentrations of organic N and the fruit yield were significantly higher in grafted plants than in non-grafted controls. These results were ascribed to differences in N utilization and assimilation efficiency between grafted and non-grafted plants. Pulgar et al. (2000) conducted a similar study using watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai, cv. 'Early Star'], which was either non-grafted or grafted onto the rootstocks 'Brava', 'Shintoza' and 'Kamel'. The grafted watermelon plants exhibited significantly lower NO_3^- concentrations, accompanied by higher NRA and higher concentrations of total-N, free amino acids and soluble proteins in comparison with non-grafted ones. Enhancement in the leaf N concentration was reported also for mini-watermelon plants (cv. 'Ingrid') grafted onto the commercial rootstock 'PS 1313' (*C. maxima* Duchesne × *C. moschata* Duchesne) and grown under open field conditions (Rouphael et al., 2008b) in comparison with non-grafted plants. On the other hand, xylem sap collected from the decapitated stem base of melon plants cv. 'Khatooni' grafted onto the rootstock 'ShintoHongto' contained more NO_3^- than the sap from self-rooted plants (Salehi et al., 2010). Combined consideration of these results leads to the conclusion that the tested *Cucurbitaceae* rootstocks enhance not only the uptake of NO_3^- and its translocation to the shoot but also its utilization by the plant through a more intensive assimilation into amino acids and proteins. This consideration was supported also by recent results of Colla et al. (2010a). These authors found that hydroponically-grown melon (*C. melo* L.) plants grafted onto two *C. melo* rootstocks ('Dinero', 'Jador'), and a *Cucurbita* hybrid rootstock ('P360') needed 5.7, 5.2, and 6.1 mM of NO_3^- in the nutrient solution, respectively, to reach half-maximum shoot dry weight, whereas ungrafted plants and plants grafted onto the *Cucurbita* hybrid rootstock 'PS1313' needed 13.1 and 9.1 mM of NO_3^-, respectively. The high nitrogen use efficiency in melon plants grafted onto the rootstock 'P360' was confirmed in a field experiment where marketable yield, N use efficiency, and N uptake efficiency increased by 9%, 11.8%, and 16.3%, respectively, when 'Proteo' plants were grafted onto 'P360', in comparison with ungrafted 'Proteo' plants (Colla et al., 2010a).

The higher nitrogen uptake efficiency of some graft combinations can minimize or even eliminate yield losses owing to marginal soil fertility. Furthermore, using grafting and breeding to develop commercially acceptable varieties with enhanced nutrient use efficiency by improving morphological, biochemical, and chemical traits may be considered as new strategies to restrict nitrogen loss due to leaching (Simonne et al., 2010).

Beside nitrogen, phosphorus uptake also seems to be enhanced by grafting onto some rootstocks. On the other hand, phosphorus uptake can be reduced by grafting, depending mainly on the genotype of the rootstock (Kawaguchi et al., 2008). This was reported for melon (*C. melo* L.) grafted onto pumpkin (*C. moschata* Duch.) 'No. 1 Shengzhen' (Qi et al., 2006). Mineral nutrients are absorbed by the roots and transported in the xylem sap to the upper part of the plant. In agreement with this consideration, the low concentrations of P observed in the scions of tomato/pepper and pepper/tomato grafts were ascribed to the smaller root system and restricted xylem hydraulic conductivity from the rootstock to the scion owing to low compatibility (Kawaguchi et al., 2008). On the other hand, a limited assimilate supply from the scion to the rootstock could have also contributed to reduced root size, thereby decreasing the mineral nutrient concentrations in the scions of the tomato/pepper and pepper/tomato grafts (Kawaguchi et al., 2008). In contrast, higher P concentrations in the leaves of grafted plants, or higher translocation rates from root to shoot, in comparison with non-grafted plants have been reported by Leonardi and Giuffrida (2006) for eggplant grafted onto 'Beaufort', Rouphael et al. (2008a) for cucumber grafted onto 'Shintoza', Colla et al. (2010b) for watermelon grafted onto 'P360' and 'PS1313' (*C. maxima* Duchesne × *C. moschata* Duchesne), and Salehi et al. (2010) for melon grafted onto the *Cucurbita* rootstock 'ShintoHongto'. Due to the low mobility of P, a more vigorous root system characterized by a higher density of root hairs is expected to increase active P uptake by the plants. Beside the root structure, other mechanisms governed by the root system may also be involved in the enhancement of P uptake by grafted fruit vegetables. Indeed, as reported by Colla et al. (2010b), the uptake of P by watermelon grown on pumpkin rootstocks ('P360' and 'PS1313') was facilitated by exudation of organic acids by the roots. Moreover, Gent et al. (2005) conducted a hydroponic experiment using two different subspecies of summer squash (*Cucurbita pepo* L.) and reported that depletion of P in nutrient solution resulted in exudation of more citric and succinic acid, especially in *C. pepo* spp. *pepo*. The exudation of organic acids, particularly citric acid in response to P depletion, was associated with the ability of the plant to accumulate more inorganic nutrients (P, K and Zn) when grown in the field.

Enhancement of K uptake due to grafting has been also reported by some authors, specifically Leonardi and Giuffrida (2006) for eggplant grafted onto 'Beaufort' and two *S. lycopersicum* rootstocks ('PG3' and 'Energy'), Qi et al. (2006) for melon grafted onto 'No. 1 Shengzhen' (*C. moschata* Duch.), Rouphael et al. (2008b) for mini-watermelon grafted onto pumpkin ('PS 1313'), Zhu et al. (2008) for cucumber seedlings grafted onto 'Chaojiquanwang' (*C. moschata* Duch.), and Albacete et al. (2009) for tomato grafted onto three high-vigour rootstocks derived from a cross between *S. lycopersicum* L. var. cerasiforme × *S. cheesmaniae* (L. Riley) Fosberg. The mechanisms underlying the increase of the leaf K concentration in the above referenced graft combinations are still unclear. However, since the K mobility in the soil is based predominantly on diffusion, a more vigorous root system is expected to improve the total K availability in the rhizosphere and concomitantly its uptake rate by the plants. Nevertheless, a recent report of Albacete et al. (2009) points to a more complex mechanism operating at biochemical level in the K-efficient rootstocks, which is controlled by cytokinins synthesized in the root.

Enhanced Ca uptake due to grafting and higher Ca translocation rates to the fruit are important in fruiting *Solanaceae* because of the high susceptibility of their fruit to the calcium-related disorder "blossom-end rot" (BER). Fernández-García et al. (2004) found a significant increase in leaf Ca concentrations when the tomato cultivars 'Fanny' and 'Goldmar' were grafted onto the tomato rootstock hybrid 'AR-9704'. Similarly, Leonardi and Giuffrida (2006) found significant increases in the leaf Ca concentrations of tomato plants grafted onto the interspecific rootstock 'Beaufort'. The leaf Ca concentration increased also in eggplant grafted onto the rootstocks 'PG3', 'Energy', and 'Beaufort' (Leonardi and Giuffrida, 2006). However, these papers do no report the impact of grafting on the incidence of BER. Recent data indicate that the incidence of BER depends on the vigorousness of the rootstock but also on the rootstock/scion combination. A very vigorous rootstock such as 'Maxifort' seems to increase the incidence of BER when the scion is 'Classy' and decrease it when the scion is 'Brigeor' (Schwarz and Krumbein, unpublished). Furthermore, the differences in BER incidence between grafted and non-grafted plants disappear, when 'Piccolini' is used as a scion. Unlike tomato, the fruit vegetables of *Cucurbitaceae* do not exhibit any increase in their ability to take up Ca when grafted onto some commonly used rootstocks as indicated by results reported by Rouphael et al. (2008a) for cucumber grafted onto 'Shintoza', Huang et al. (2010) for cucumber grafted onto 'Black Seeded' and 'Chaofeng Kangshengwang', Edelstein et al. (2005) for melon grafted onto 'TZ-148' and Rouphael et al. (2008b) for mini-watermelon grafted onto 'PS 1313'. The impact of grafting on Mg uptake depends largely on the rootstock genotype. Some tomato rootstocks such as 'He-Man' may decrease the leaf Mg concentrations (Savvas et al., 2009). However, other rootstocks seem to increase significantly the Mg uptake, such as 'Energy' when it is used to graft eggplants (Leonardi and Giuffrida, 2006) and 'PS 1313" when it is used to graft mini-watermelon (Rouphael et al., 2008b).

Most of the above-referenced instances of enhanced K, Ca, or Mg uptake by some graft combinations originate from experiments, in which the supply of the corresponding nutrients was normal. Hence it is not clear if the enhancement in K, Ca, or Mg uptake by the above-referenced graft combinations would occur also under conditions of a limited supply and if this enhancement in uptake would be beneficial in terms of yield performance. Thus, further research is needed to establish causal relationships indicating higher yield performance due to improved uptake of K, Ca, or Mg by grafting when the availability of these nutrients is inadequate, in comparison with non-grafted plants grown under the same nutrient status.

In most cases, grafting of fruit vegetables either decreases or has no effect on the uptake of micronutrients (Edelstein et al., 2005; Rouphael et al., 2008a; Savvas et al., 2009; Huang et al., 2010). However, some rootstocks increase the efficiency of grafted plants to take up and translocated Fe and/or Mn to the shoot, in comparison with the corresponding self-rooted cultivars (Rivero et al., 2004; Colla et al., 2010b; Huang et al., 2010). Low availability of metallic cations in crops of fruit vegetables, which is associated with the occurrence of chlorosis symptoms resulting in concomitant yield losses, is expected mainly in soils with excessively high pH. The responses of grafted plants to stress caused by excessively high pH levels in the root environment are presented in the next heading. Thus, more information regarding the possibilities to combat stress caused by inadequate availability of Fe and other metallic micronutrients in the root zone is given in the next heading.

5. Alkalinity tolerance

Alkalinity in irrigation water and soils restricts the cultivation of plants in many areas of the world, and especially in the Mediterranean basin. Alkaline water and soils are generally characterized by low bioavailability of plant nutrients, and high levels of insoluble $CaCO_3$ in the soil and HCO_3^- in the soil solution. The concentration of HCO_3^- interacts strongly with the availability of several micronutrient ions, especially Fe^{2+}, and it is often considered to be the primary factor causing chlorosis in cultivated plants, which may lead to serious yield losses. In a recent study, Colla et al. (2010b) found substantial differences in the agronomical, physiological and biochemical responses between grafting combinations of watermelon plants, cv. 'Ingrid'. In particular, the watermelon plants were either ungrafted or grafted onto two pumpkin [*Lagenaria siceraria* (Mol.) Standl.] rootstocks ('Macis' and 'Argentario') and two bottle gourd (*C. maxima* Duchesne × *C. moschata* Duchesne) rootstocks ('P360' and 'PS1313') and exposed to two levels of nutrient solution pH, specifically 6.0 or 8.1 dS m^{-1}. The leaf chlorosis symptoms in the plants grafted onto bottle gourd rootstocks, and the ungrafted plants were, in general more pronounced than those in plants grafted onto pumpkin rootstocks. Plants grafted onto pumpkin rootstocks and exposed to an excessively high external pH level were capable of maintaining higher net assimilation rates, and exhibited a greater strong capacity to accumulate Fe in the aerial part, and a better plant nutritional status (higher P and Mg) in the shoot tissue in comparison with those grafted onto bottle gourd rootstocks and the ungrafted plants. The above responses of watermelon plants grafted onto pumpkins to high pH levels were associated with a higher exudation of organic acids (citric and malic acids) by roots, which presumably facilitated the uptake of nutrients by the plants.

Excessive soil acidity may be a problem for crop plants in many areas of the world (Haug, 1984). However, to date there are no reports in the international literature on the response of grafted plants to the rhizosphere pH and thus, research in this field is currently an urgent need.

6. Conclusions

Grafting of fruit vegetables is expected to expand as an alternative means to combat soil-borne pathogens, and for several other reasons which are covered in other papers of this issue. In part of this review we have focused on the use of grafted plants in commercial practice to minimize the toxicity of heavy metals to plants, and restrict their concentrations in the edible fruit. Furthermore, we report on the prospects of gaining benefit from many rootstocks that can restrict or enhance the uptake of specific nutrients, in comparison with non-grafted cultivars of fruit vegetables. These rootstocks can be used to minimize the impact of inadequate or excessive nutrient levels in soils. Rootstocks with a higher efficiency to take up nutrients require less fertilizer and may decrease pollution of the environment. The responses of grafted plants to deficient or excessive external concentrations of nutrients and heavy metals depend on the scion as well as the rootstock genotype, and the rootstock-scion physiological interaction. These responses also depend on biotic and abiotic conditions prevailing in the field. Hence, the response of any particular rootstock-scion combination grown in a specific environment cannot be generalized to include all graft combinations obtained using this rootstock, or to crops grown under distinctly different conditions.

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