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Review Article

Studies of Grafts in vegetables, an alternative for agricultural production under stress conditions: Physiological responses

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Abstract

Vegetable production by grafting is a technique which it has made possible to resume agricultural soils which previously could not be produced due to stress generated by various abiotic factors, like a lack of water, stress by high or low temperatures, and or heavy metal contamination, among them. It has been documented and defined a number of graftings which they are tolerant to different factors; however, when it comes to auscultating information related to understand the molecular responses and observe what are the biochemical changes and physiological responses of grafted plants, it is dispersed. The current paper attempts to provide basic information documented on technique, addressing the molecular, biochemical and physiological responses, and thus get a clear perspective on the use of grafts, making this practice be used with most frequently by all its advantages.

Introduction

The first records of grafts showed that it was a long time in fruit trees [1]; During the 20s, the technique was used successfully in some vegetables [2], but it was not until the 21st century, the interest in its use for the production of large-scale crops [3]. The concern for the environment led to the search for alternatives in the integral production of crops, and one of them was the use of grafts. Currently there are some seed companies that have dedicated themselves to obtaining both improved crops and grafts. However, even though the advantage of the use of grafts is well known, several factors must be considered in order for the grafting technique to have the expected results. On the other hand, at present the tendency of the population is to consume more horticultural products, making the agricultural sector can not meet this demand without the use of large quantities of agrochemicals [4]. Along with this, the planting of products out of season and the use of land infested by phytopathogenic microorganisms generate a stress for the plants making frequent an imbalance in the normal development of the crops making necessary the use of agrochemicals and becoming an uncontrolled cycle that it damages the environment and generates several physiological disorders in plants and deterioration of the quality of the products consumed. For all this, is that the use of grafts has become an alternative to maintain the demand for horticultural products in many parts of the world.

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Purpose of the grafts in vegetables

The main objective of the technique is the use of materials tolerant to diseases such as grafts, these materials make possible the production in soils where it would not normally be possible to normally. In some countries it is a widely used technique, although in the Occident it is a procedure recently introduced to the agricultural area. [5]. However, other important factors such as lack of water or the use of crops that tolerate different types of stress [6,7], make it necessary to search for alternatives such as grafts [8,9].

The grafting technique is usually done by hand, on the other hand, there is currently specialized equipment that is responsible for performing the grafts automatically. There are several types of grafts that, depending on the varieties to be grafted, the type of acclimatization chamber with which it is counted and the purposes for which the plant is wanted; the type of graft must be chosen. In figure 1, different variations of the technique for Solanaceae grafting are shown. Different technologies and procedures have been documented for other types of vegetables, which are described in the works of and [3,10].

This procedure has been used with great success for the control of diseases caused by fungi such as Fusarium oxysporum, Sclerotium rolfscii, Verticillium; wilting caused by bacteria such as Ralstonia solanacearum and some nematodes of the genus Meloidogyne spp. Among others [11-14]. However, the success of the technique for the management of diseases depends on a large number of factors; from the individual tolerance of grafts, the combination of variety- grafts and some other elements such as the genetic variability of plants [15,16].

Although these interactions and disease tolerance mechanisms are not yet fully understood, it is believed that the main tolerance is provided by the natural resistance of the grafts used. However, in studies where double-rooted grafts have been carried out, a partial tolerance of the grafted varieties is shown, suggesting that in the interaction between variety-grafts, associated substances are transported in defense against diseases such as *Fusarium*, said substances are synthesized in the root of the rootstock and transported via xylem to the variety [3,17]. Another of the main advantages of the use of grafts is the improvement in crop yields; Khanh et al. [18],

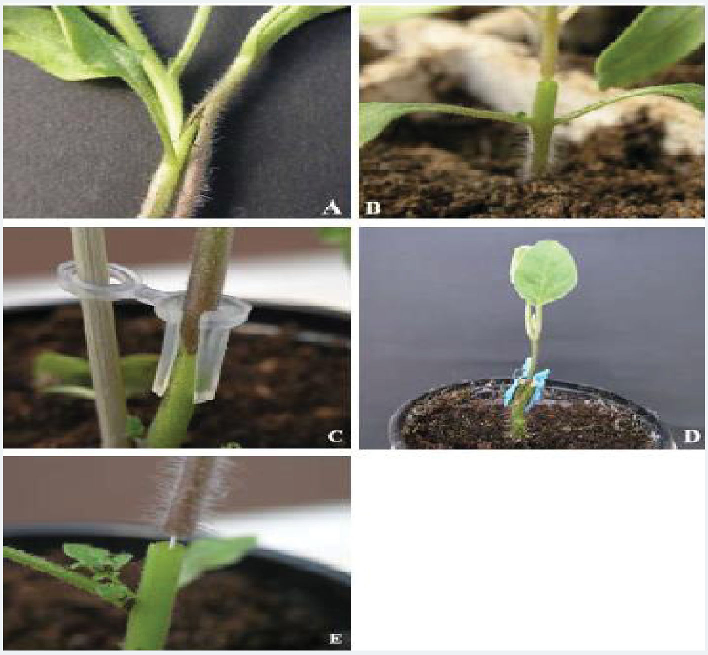

Figure 1: Grafting methods performed in Solanaceae; **a)** Double root approach method; **b)** tube insertion method; **c)** method of attachment; **d)** barb method; **e)** side pin insertion method. Bletsos and Olympios [61].

observed that tomatoes grafted on two hybrids as grafts had greater vigor than non-grafted varieties, in the same way they observed that the grafted plants had a higher yield ranging from 11 to 38% compared to non-grafted treatments. Similar results, but in watermelon, were obtained by Alan et al. [19], who observed a significant increase in the yield of grafted watermelon cultivated in two different environments, determine that under their study conditions the grafted watermelon not only increases agronomic parameters, but in the same way the technique does not affect the final quality of the watermelon fruit; this affirmation differs from that of Mohamed et al. [20], who also observed an increase in the yields of grafted watermelon. However, indicate that the fruit quality, especially the lycopene content in fruit, is modified under the conditions in which it was developed. The technique has also been used for other purposes such as improving the quality of grafted plants, greater absorption and intake of water and nutrients, tolerance to certain types of abiotic stress, tolerance to contamination by heavy metals, among others [21-24].

However, even knowing the advantages of its use, the main disadvantage of the technique of grafting, could be considered the economic factor, the use of tweezers or bands for gluing, knives, disinfectants, gloves, additional substrate, conditioning an acclimatization chamber , seed of the grafts (commercial hybrids), labor and other indirect factors cause the production costs of the grafted plant to rise [5], which in some cases could result in a possible disadvantage compared to the traditional production. Other factors to be considered as disadvantages may be the use of skilled labor, the lags in the production times, the initial delay caused by the grafting time and finally the limitation of the use of certain grafts.

Physiological, biochemical and molecular responses of grafted plants

The physiological responses of the grafted plants are given in the same way as in a normal plant, that is, they depend on factors such as the type of stress, duration of the same and intensity or progress of this, likewise it also depends on the genotype of the varieties, the state of development of the plants and their interaction with the environment [25,26].

Changes in the morphology of the grafted plant

Undoubtedly one of the most important aspects in grafted plants are the morphological modifications resulting from the grafts-variety interaction. Recently it was observed that grafted plants of beans had similar characteristics to non-grafted ones, however, factors such as plant height, number of leaves and flowers increased in plants grafted 28 days after the graft (DDI) Bernal-Alzate et al. [27],these modifications can be mediated by the production of growth regulators from the root transported via xylem to the aerial part of the plant [28,29]. On the other hand Kudo and Harada [30], observed how the morphology of the leaves of potato plants were modified due to the transport of RNA from the tomato rootstock towards the aerial part, determining that the amount of genetic material transported was enough to modify size, shape and quantity of trichomes in the leaves of grafted plants, these and other types of morphological changes have been documented by other researchers [31-33].

Stomatal conductance and CO_2 assimilation

Along with cell growth, photosynthesis is a primary process affected by lack of water and salinity, the effects on the plant range from a decrease in the assimilation of CO_2 caused by poor transport through stomata and mesophyll, to alterations in photosynthetic metabolism or even can lead to side effects causing oxidative stress in the cells [34-36], in a study conducted by Yang et al. [37], in plants of Lagenaria siceraria autografted and grafted on a rootstock tolerant to high salinity, it was observed that when increasing stress due to high salinity the stomatal conductivity (Gs) and the intracellular CO_2 concentration (Ci) decreased in both. However, those grafted on the tolerant rootstock had higher Gs and Ci, indicating that probably stomatal closure was initiated by a signaling from the tolerant rootstock; These results are similar to those obtained by Rouphael et al. [38], who indicate that in both melon and grafted cucumbers there is a decrease in the photosynthetic activity of the plants. However, this decrease is up to 50% higher in plants that are not grafted and are under stress conditions due to high concentrations of NaCl; the same authors [38], found an inverse linear correlation of the photosynthetic activity with respect to the concentration of Na + and Cl- in leaves, attributing the excess of these to a disorder in the photosynthetic apparatus; similar studies witnessed the same photosynthetic activities [39,40]. Under normal conditions (without stress), grafted plants behave in a similar way to previous studies; in the case of Amaro et al. [41], observed that the grafting of the cucumber plants increased the stomatal conductance and photosynthetic capacity of the plant compared to those that had not been grafted; Similar results were reported by Liu et al. [42], attributing this increase in photosynthetic capacity to an increase in the amount of chlorophyll a and b in plants; additionally they reported the increase in carbohydrate accumulation in fruit, due to the activity of two key enzymes for this process in melon plants; Similar results were presented by QI et al. [43] and González et al. [44], in melon and citrus respectively. Likewise, Qinghai Gao et al. [45], Suggest that grafted plants have the ability to better use Cl under stress conditions, as well as presenting a better photosynthetic efficiency compared to those plants that were not grafted, this higher photosynthetic efficiency may be due to the fact that high levels of Cl decrease the transpiration of the leaves, improving the efficiency of water use in photosynthesis [46].

Enzymatic activity in grafts

When plants are under some kind of stress or when at some phenological stage of the crop occurs, one of the main indicators of these changes is the activity of certain enzymes. In grafts studies have been conducted to determine the activity of these enzymes. Some researchers have concentrated on observing that physiological and / or biochemical processes occur in different stages of the graft, it has been found that during the process of sticking the graft in incompatible plants there is an accumulation of ERO'S (Reactive oxygen species) [47-49], these in turn degrade the RUBISCO and therefore there is a decrease in the photosynthetic capacity of the plant specifically in carbon fixation [50]. Inefficiency in photosynthetic capacity leads to an incompatible plant death. Liao et al. [51], reported that the grafted promoted the increase in the concentration of key enzymes for RUBISCO photosynthesis (Ribulosa 1,5 bisphosphate Carboxylase-Oxygenase) and RCA (RUBISCO activasa), these enzymes play an important role that directly affect the photosynthetic potential of the plants and therefore disrupt their yield potential [52]. Different types of response occur in grafted plants such as the accumulation of ROS, production of secondary metabolites directly or indirectly involved with the elimination of pathogens, synthesis of nitric oxide, and hypersensitive responses [53]. In the recent decade, studies have been carried out showing an increase in enzymatic activity and other biochemical compounds of grafted plants against various types of stress, Table 1 summarizes these studies.

Table 1: Enzymatic activity and other biochemical compounds of grafted plants against various types of stress.

Enzymes	Grafted plant	Condition of stress	Behavior of the enzyme	Reference
Catalase (CAT), Peroxidase (POD), Superoxide dismustase (SOD).	Tobacco	potato virus Y^{NTN}	Increase in POD and SOD, Decrease in CAT	[62]
Subunit 1 and 2 of the CAT isoenzyme	Tomato	*Fusarium* spp.	Presence of Subunits	[63]
CAT, SOD	Tomato	Water stress	SOD and CAT increase	[64]
Phenylalanine ammonium lyase (PAL)	Chili	*Fusarium* spp.	Increase at the beginning of the infection, stabilize or decrease days later.	[65]
Phenylalanine ammonium lyase (PAL)	Eggplant	*Verticillium* spp.	Increase PAL	[66]

Molecular responses of grafted plants

All physiological and / or biochemical responses are initialized by an expression of a specific gene. Work has been done on different types of grafts subjected to different types of stress to determine which specific genes are expressed and what is the biochemical or physiological response of these in grafted plants. It has been amply demonstrated that genetic exchange occurs in plants grafted differently, Crété et al. [54], establish that one of the factors for the exchange of silencing signals depends partially on the type of graft used, in their work they describe how using three different techniques of grafting only the technique of tube grafting (where a part of the variety wedge-shaped inserted in a ring cut on the rootstock), resulted in grafts systematically silenced by a sign of the rootstock; in 1997 Palauqui et al. [55], propose that the systemic silencing of genes in grafts occurs even when the expression of the silenced gene is not given in both varieties (rootstock and variety), these results do not agree with those reported by Shaharuddin et al. [56], who demonstrate that in tomato the exchange of messenger RNA (mRNA) between grafts-variety occurs only when the expression of a silenced gene is over expressed in both varieties before grafting. Wang et al. [57], found that the criollo varieties grafted tomato on a transgenic (which expresses the gene repressor MhGAI1 in the route of synthesis of gibberellins), accumulated transcripts of the gene MhGAI; these plants presented dwarfism, however, produced a greater amount of solids soluble in fruit, sugars and organic acids compared to those that were not grafted suggesting that the signaling for the synthesis of gibberellins, affected the quality of the fruit of the grafted plants. On the other hand, Ntatsi et al. [58], observed the tendency of accumulation of abscisic acid (ABA) in tomato plants was higher in grafted plants compared to non-grafted plants, regardless of the rootstock used in their study, attributing that the gene reviewed (LeNCED1), did not participate in the synthesis of ABA.

In a different work Jiménez et al. [59], observed that the peach rootstock tolerant to salinity when subjected to this stress on expressed the specific gene P5SC at the same time that there was an increase of sorbitol, raffinose and proline determining that this gene could be used as a response marker to water stress in peach plants. One of the most recent studies on vegetables to date is that of Miao et al. [60], who based on the fact of the genetic transfer of the rootstock towards the variety, tested if there was a gene silencing related to the infection of the cucumber mosaic virus (CMV Cucumber mosaic virus); they tested a transgenic rootstock resistant to the virus and a susceptible variety, finding that the gene silencing provided by the resistance to this virus was transmitted by the rootstock towards the variety; the same work concluded, that these plants may remain undetectable for gene markers of transgenic resistance and, may provide new approaches to evade concerns with biosecurity over genetically modified organisms (GMOs).

Conclusions

The use of grafts is a relatively new technique in the agricultural area, the increase of its use is due in large part to its multiple advantages outweigh the economic factor that entails. That is why currently it is necessary to focus part of the research in this area to the search of Creole grafts that reduce the costs of the technique; This search should be conducted in finding materials not only that lead to increase the productive potential of the plants, but also, the grafts obtained must be tolerant to some type of stress. It is undeniable that the use of the technique represents an alternative for the production of vegetables in a way more friendly to the environment, so its use could be increased in the coming years.

On the other hand, at the present time the increasing number of researches to enlarge of knowledge in the understanding of the physiological, biochemical and molecular changes that occur in grafted plants, continues to rise and although more and more is understood about the behavior of grafts under different types of conditions; sometimes the results of these investigations may not coincide and sometimes not be conclusive.

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