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Influence of grafting method in the quality of tomato seedlings grafted and intended for commercialization

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Abstract

The effect of grafting method on parameters of tomato seedlings was studied in greenhouse conditions. Cleft, Tube and Hole grafting methods were experienced on Meryl F1 tomato seedlings. Controls non-grafted were used in parallel. Forty five days after sawing, parameters were measured on each cultivated tomato plant. Thus percentage of grafting success, number of seedling leaves, seedling length, wet weight of seedling, dry weight of seedling and dry matter ratio were determined. The study shows that all methods of grafting tested gave high success rates. The tube grafting method was superior to the other grafting methods in the global parameters tested. The cleft and tube grafting methods were compared in the total studied parameters. The hole grafting method was delayed in all studied parameters. Tube grafting is therefore considered an important technique for tomato seedlings production when the conditions of grafting are perfectly controlled.

Keywords

Cleft grafting, Tube grafting, Hole grafting, Tomato seedlings, Commercialization seedlings parameters.

Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the *Solanaceae* family and is currently one of the most important vegetable crops. Tomatoes spread all over the world, in the cold, temperate and warm regions. The fruit is an essential part of daily alimentation, and consume either fresh, cooked or processed, which is characterized by its high nutritional value as it contains carbohydrates, mineral salts, vitamins and organic acids of great nutritional importance.

Tomato ranks third among vegetables planted behind potatoes and sweet potatoes, it grows globally on an area of 5 million hectares, and annual production of up to 170 million tones and yield of 34 tones/ha. Locally, tomato's crop ranked first among various vegetables developed in the country, which is grown on an area of 12 thousand hectares, and annual production of 670 thousand tons and yield of 56.5 tones/ha (FAO, 2014).

Indoor tomato agriculture gets importance through its excellence in production intensification and increased land investment, especially after population increasing and poverty of food sources. Therefore, it's imperatively to find new means to increase agricultural production and food security. As a consequence of tomatoes intensive and repeated farming, especially in greenhouses, it increased the attack risk by various diseases and pests, which play a crucial role in the decline of production and plants death. Researches have confirmed that tomatoes exposed to infection by more than 200 pathogens in the world, and half of this number is originally of the soil that infect roots. The most important soil-borne diseases are Fusarium and verticillium wilt, root rot, croky root, bacterial wilt, root-knot Nematodes, ToYLC virus and broomrapes (Collonier et al., 2001; Draie, 2009).

The solution to many of problems faced by growing vegetables in general and tomatoes in particular, and for improving the productivity of vegetable crops so as to meet the rising demand for consumption, grafting technology has introduced in the production of different vegetable crops. This technique has become a prerequisite in organic farming and sustainable agriculture (Rivard, 2006).

Hence the importance of our research, which is to focus on vegetable grafting technique and to work on finding the best approaches convenient, that elevate this technology in order to achieve higher yields and optimum conditions for use. The process involves union of two parts (a rootstock and scion) together from two different plant parts to form a single, living plant (Kumar, 2011).

Grafted vegetables were planted for the first time in Japan and Korea at the end of 1920th, when watermelon was grafted to squash rootstocks, while eggplant have been grafted onto *Solanum integrifolium* in the fifties of the last century (Leonardi, 2016). Since, cultivation of grafted vegetables has increased significantly and became essential technology in the production in Korea, Japan, and some countries in Asia, Europe, and which is characterized by the intensive use of soils (Kubota, 2008; Bekhradi, 2011). This technique has been adopted in Mediterranean as an alternative to chemical for soil treatments aiming to control the soil-borne pathogens, to increase the productivity of crops and in addition to be considered obligatory application in organic farming (Rivero et al., 2003; Rivard and Louws, 2008).

According to Lee et al. (1998), 450 million grafted plants in Korea and 750 million grafted plants in Japan (Equivalent to 81% and 54% of the vegetable plants in each of the two countries) were produced this year. The number of tomato plants in Spain between 2000 and 2004 has increased from less than 1 million to more than 45 million plants (Besri, 2005). A similar data is cited in Italy and France. In Greece, the proportion of grafted watermelon plants is more than 90% of the cultivated watermelon plants, while the grafted melon plants are 50% of the total cultivated melon plants, but the tomatoes, eggplants and cucumbers are still lower (Traka-Mavrona et al., 2000). In USA, grafting is an essential condition in the cultivation of vegetables, because it is very important in resistance to soil-borne pathogens, improves productivity and quality, and most importantly it is an inevitable measure in the organic and successive crops of tomatoes (Khah et al., 2006; Rivard, 2006; Kubota, 2008; NARO, 2011; Leonardi, 2016; Ashok and Sanket, 2017; Kyriacou et al., 2017).

In the Arab world, the spread of this technology is increasing, especially in Morocco, Jordan, Lebanon, Egypt and Syria. In Morocco, the proportion of tomato plants grafted is more than 25% of the total cultivated tomato plants, which is useful mainly to the resistance of soil-borne pathogens as well as to prolong the growth period of the crop (Besri, 2003; Besri, 2005) .

The importance of vegetable grafting is to increase the growth strength of the grafted plants and thus gives maximum productivity during the maximum production period. Vegetable grafting is also a powerful alternative to chemicals used to resist soil-borne pathogens. At the same level, the grafting has demonstrated its ability to resist abiotic stresses such as extreme temperatures, high soil moisture, salinity … etc (Lee and Oda, 2003; chang et al., 2008; Buller et al., 2013).

Vegetable grafting allowed to increase production, expressed in the number and size of fruits and compared with non-grafted plants, in the watermelon plant (Yetisir and Sari, 2003), cucumber (Pavlou et al., 2002), tomato (Pogonyi et al., 2005), eggplant (Passam et al., 2005).

Grafting is effective for overcoming environmental stresses including salinity (Estan et al., 2005; Ruiz et al., 2005; Colla et al., 2006), extreme temperatures (Abdelmageed et al., 2004) Black et al., 2003), and excessive soil moisture (Black et al., 2003).

Vegetable grafting has also shown great effectiveness in resistance of many soil-borne pathogens such as fungi, bacteria, viruses, nematodes and others. It has proven to be very effective in resisting fusarium wilt disease on watermelon (Rivero et al., 2003) and root fusarium wilt disease on tomato (Rivard, 2006). Grafting has also been shown to be very effective in resisting verticillium wilt on melon (Bletsos, 2005), eggplant (Ioannou, 2001), tomato (Rivard, 2006) and potato (Tsror and Nachmias, 1995). Root-rot disease (Upstone, 1968) and fibrous root-rot disease (Bradley, 1968) on tomato, were also treated using grafting.

Grafting technique has proven effective as an alternative to chemical pesticides in the resistance of viral tomato leaf disease (Rivero et al., 2003), bacterial wilt disease in *Solanaceae* family (Tresky and Walz, 1997), nematode control (Rahman et al., 2002) and broomrape control (Draie, 2009).

Not too long ago, soil sterilization with chemical pesticides was the most common method of controlling soil pests. Because of the problems caused by these chemicals, both to the safety of the environment and to human health, their use, especially methyl bromide, has been banned. In addition to the high costs of soil sterilization, and the increasing demand for organic agriculture. The use of chemical pesticides was not possible, and it was necessary to find new safe alternatives to solve the problem of infection by various soil-borne pathogens. The grafting of vegetables was one of the best solutions available so far (Ashok and Sanket, 2017).

There are several methods of vegetables grafting currently used, including: convergence grafting (Lee, 1994; Grigoriadis et al., 2005), tube grafting (Oda, 1995; Lee and Oda, 2003), cleft grafting (Oda, 1999), vertical and lateral hole grafting (Salehi et al., 2008). All these methods of grafting are suitable for vegetable plants, especially tomatoes, with a success rate ranging from 80-100%, provided that the ideal conditions for successful of grafting are guaranteed (Kacjan-Marsic and Osvald, 2004).

A variety of problems are normally linked with grafting and producing grafted seedlings. Most important problems are the labor and techniques essential for the grafting process and after graft handling of seedlings for quick healing for 7 to 10 days. An expert can graft 1200 seedlings per day (150 seedlings per hour), but the numbers vary with the grafting method. Similarly, the post graft handling method depends mostly on the grafting methods (Ashok and Sanket, 2017). Further inventions are mechanized and robotic grafting has given a fillip to this novel eco-friendly approach (Bandey and Rai, 2003).

Importance and objectives of the research

The importance of research is to test the best way to grafting vegetable seedlings in general and tomatoes in particular, as a basis for the grafting process, which has become an essential condition in the production of seedlings for organic farming. To the extent that we succeed in choosing a typical method of grafting, it will positively affect the quality of the grafted seedlings and therefore the growth and development of the plants and the productivity resulting from the use of the seedlings. The choice of the ideal method of grafting reduces the loss in the time required for seedlings growth and thus enables the seedlings to obtain the desired growth stage in a relatively shorter time.

The research objectives are summarized in the study of the grafting method effect in the quality of tomato seedlings grafted, and choose the best grafting method for the production of tomato seedlings prepared for commercial marketing, ensuring the relative benefit for the producer and farmer.

Materials and research methods

1. Plant material:

The grafting was carried out on Meryl F1 tomato seedlings, obtained from S.A. Company.

2. How to implement the experiment:

Scions and rootstocks seeds were planted on the same date, in 2 cm diameter pits filled with Beat-Moss on cork trays (Figure 1). The seedlings were left to grow and develop within incubator rooms (Figure 2), where relative moisture (85%), temperature (25° C) and light intensity (800 W/m^2) were provided. Then the plants were transferred a week later to the greenhouse. After a month of farming (Figure 3), all the plants prepared for grafting were sorted according to their growth force, and then grafted according to the method selected which described later. After grafting and fixing the scion and rootstock by special clips (Figure 4), the grafted plants are placed directly into the incubator chambers. Ten days of placing the grafted plants in the incubator they were transferred to the greenhouse for acclimatization. A week later, the plants were ready to be marketed and planted in the place of permanent agriculture, where the required measurements and readings were taken.

Figure (1) Trays of culture (after two weeks of planting seeds)

Figure (2) incubating chambers (A- external view, B- internal view)

Figure (3) seedlings after a month of planting and before the grafting

Figure (4) Forms of grafting clips used to fix the scion onto the rootstock

- **Cleft grafting:**

The rootstock is cut in the form of a V and the scion is cut in the form of a reverse V. The scion is then inserted into the rootstock and installed by a special clip (Fig. 5-a).

- **Tube grafting:**

Both the scion and the rootstock are cut in a slant and at the same degree of inclination (45°), where the rootstock is cut above the cotyledons and the scion is cut at a length of 5 cm, leaving at least two real leaves. After the rootstock and the scion are cut off, their coherence was assured them with small plastic clip and the grafted seedlings were transferred into the incubator chamber (Fig. 5-b).

- **Hole grafting:**

The rootstock is cut above the cotyledons, and the scion is cut the aerial part about 5 cm long. The scion is then prepared in the form of a pointed head, while the rootstock is punctured at the top (the place of cutting). The scion is then inserted into the hole at the top of the rootstock and fixed by a grafting clip (Fig. 5-c).

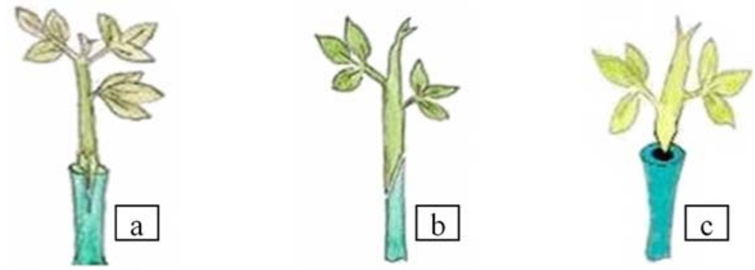

Figure (5) Methods of grafting tested (a-cleft grafting, b-tube grafting, c-hole grafting)

3. Place the experiment execution:

The experiment was carried out in a greenhouse belonging to S.A. Company in Syria (Al-Bustan Farming), so that the ideal conditions for the grafting and following the growth and development of seedlings were assured.

4. Trial design and treatment distribution:

The experiment was designed in a randomized manner (Figure 6), with four treatments distributed as follows: (Cleft grafting, Tube grafting, Hole grafting, Control without grafting).

Control	Hole grafting	Cleft grafting
Hole grafting	Control	Tube grafting
Cleft grafting	Tube grafting	Hole grafting
Tube grafting	Cleft grafting	Control

Figure (6) Design of experience of tested grafting methods

By 3 replicates per treatment, and 24 plants per replicate, so that the total number of plants used in the experiment is $4 \times 3 \times 24 = 288$ plants.

5. Measurements and readings:

Percentage of grafting success, number of seedling leaves, seedling length, wet weight of seedling, dry weight of seedling and dry matter ratio were measured 45 days after sawing for all treatments.

6. Statistical analysis:

All statistical analyzes of results were obtained by the statistical program (Sigma Stat V3.5), ANOVA analysis of variance was done to calculate the least significant difference LSD at a moral level $\alpha = 5\%$.

Results

After about two months of seeds planting (one month of the grafting), all the previously mentioned measurements were taken, and the results were as follows:

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1. Percentage of grafting success:

The results showed significant differences in the percentage of grafting success. The tube grafting method gave the lowest percentage (85%), it was exceeded by the method of hole grafting (90%), while the method of cleft grafting was the superior (95%), (Figure 7).

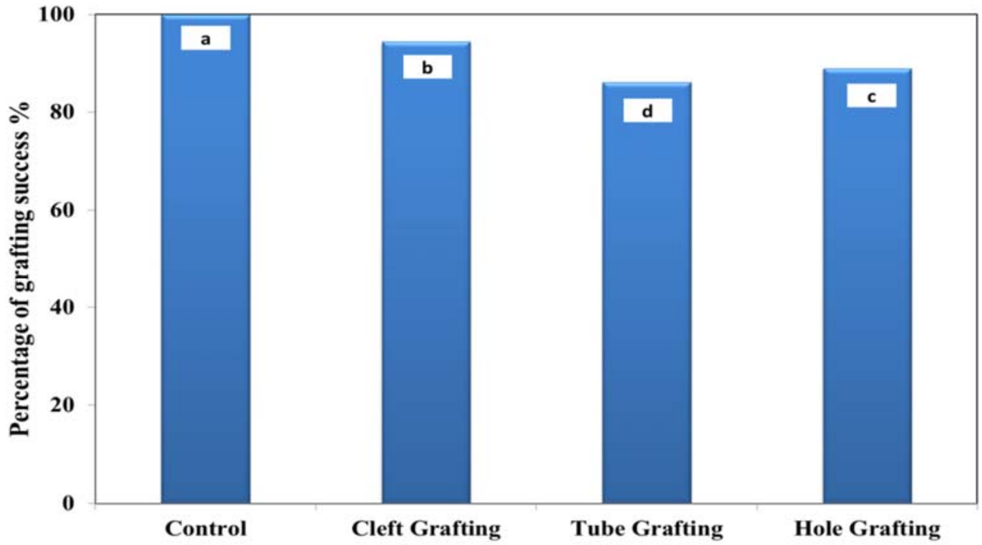

Figure (7) Percentage of grafting success (%) after 1.5 months of cultivation for the studied grafting methods and control. The values represent the mean ± SE. By grafting method, values with the same letter are not significantly different (ANOVA, α=5%, n = 24)

2. Number of seedling leaves:

The tube grafting method gave a number of leaves per seedling equal to the non-grafted seedlings, which exceeded 5 leaves. The method of cleft grafting gave results similar to those given by tube and non-grafted methods, while the method of hole grafting was significantly delayed from all other treatments, (Figure 8).

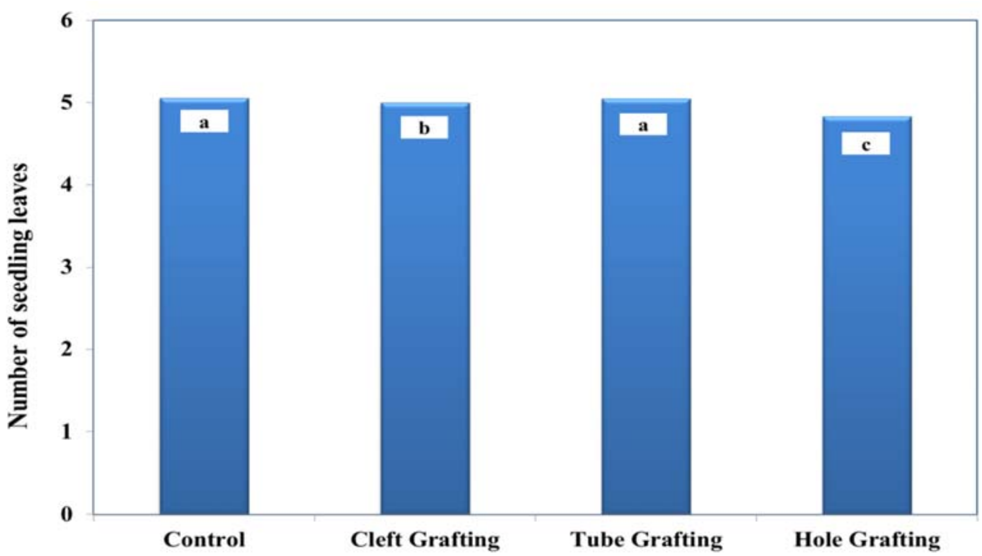

Figure (8) Number seedling leaves after 1.5 months of cultivation for the studied grafting methods and control. The values represent the mean ± SE. By grafting method, values with the same letter are not significantly different (ANOVA, α=5%, n = 24)

3. Seedling length:

The method of tube grafting was also superior to the seedlings on each of the two methods of grafting, cleft or hole grafting, so that the values were 4.1 cm, 3.8 cm and 3.4 cm respectively, while the witness was superior to all grafting treatments with a value exceeding 4.5 cm , (Figure 9).

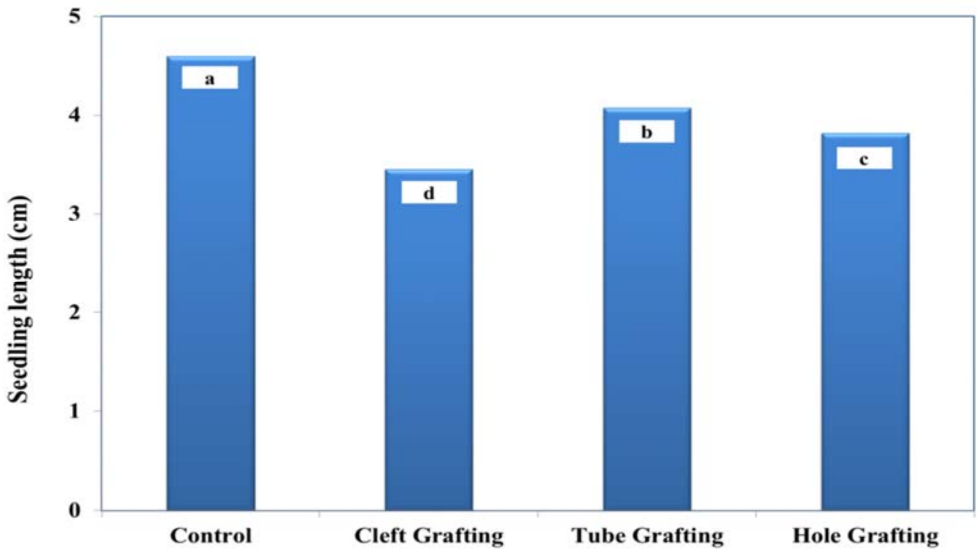

Figure (9) Seedling length (*cm*) after 1.5 months of cultivation for the studied grafting methods and control. The values represent the mean ± SE. By grafting method, values with the same letter are not significantly different (ANOVA, α=5%, n = 24)

4. Wet weight of seedlings:

The method of tube grafting showed a clear superiority in wet weight by 3.25g for each of the seedlings on the method of cleft grafting, which gave an estimated weight of 3g for the seedling, and the method of hole grafting, which gave weight about 2.9g for the seedling, while the control was significantly higher in wet weight compared with all grafted treatments and 3.6g for the seedling, (Figure 10).

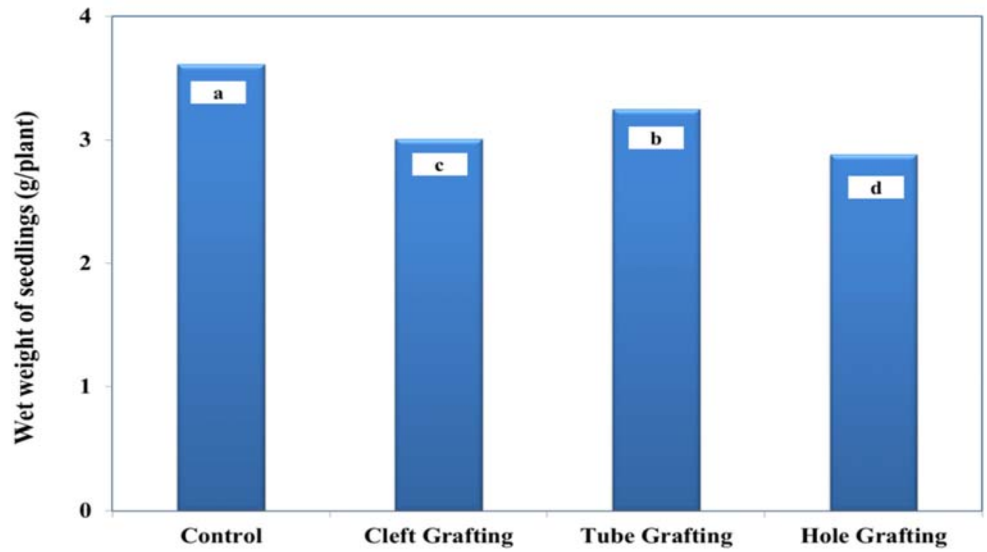

Figure (10) Wet weight of seedling (*g/plant*) after 1.5 months of cultivation for the studied grafting methods and control. The values represent the mean ± SE. By grafting method, values with the same letter are not significantly different (ANOVA, α=5%, n = 24)

5. Dry weight of seedlings:

The dry weight of the seedlings did not differ in terms of wet weight. The tube grafting method remained at the top with 0.48g per seedling followed by cleft grafting method with 0.47g per seedling. The method of hole grafting was 0.45g per seedling. While the control was the highest with 0.5g per seedling, (Figure 11).

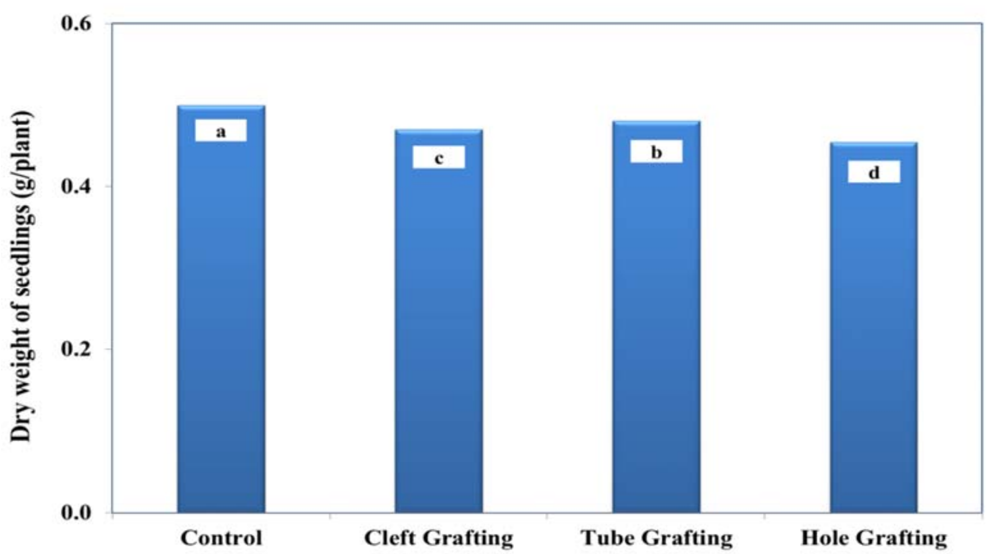

Figure (11) Dry weight of seedling (*g/plant*) after 1.5 months of cultivation for the studied grafting methods and control. The values represent the mean ± SE. By grafting method, values with the same letter are not significantly different (ANOVA, α=5%, n = 24)

6. Dry matter ratio:

The two methods of cleft and hole grafting were superior to dry matter with 16.3% and 15.9% respectively. While the tube grafting method was the proportion of dry matter in it 15.2%. However, all methods of grafting were superior to the control, which gave a lowest percentage of dry matter with 13.9%, (Figure 12).

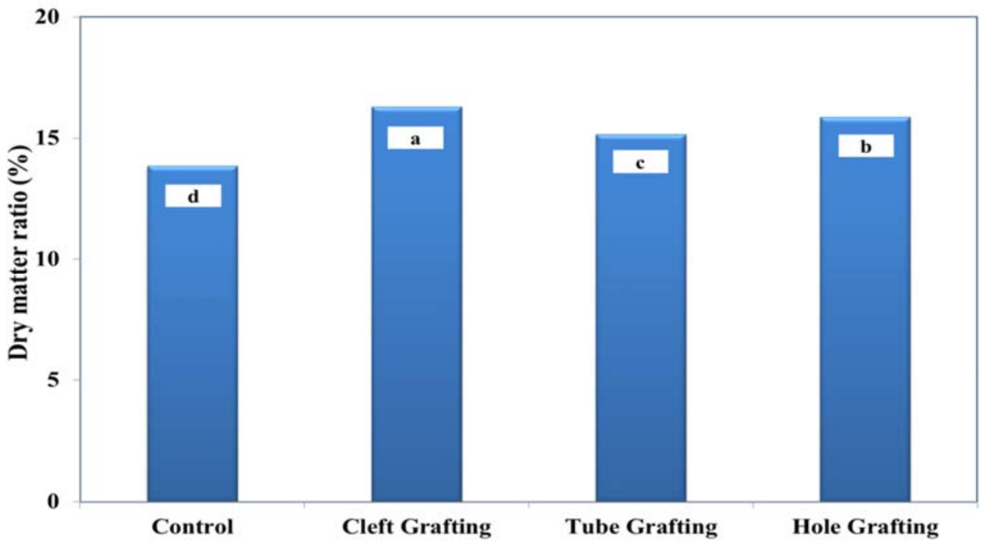

Figure (11) Dry matter ratio (%) after 1.5 months of cultivation for the studied grafting methods and control. The values represent the mean ± SE. By grafting method, values with the same letter are not significantly different (ANOVA, α=5%, n = 24)

Discussion

By analyzing the previous data we can summarize the results in Table 1. This table shows the superiority of tube grafting in four parameters which are: number of seedling leaves, seedling length, wet weight and dry weight of seedlings. While the method of cleft grafting was excelled in two parameters: percentage of grafting success and dry matter ratio, and it was the second in three other parameters so that it was parallel in the final outcome with the method of tube grafting. The method of hole grafting came third and did not excel in any studied parameters. Hence, we can say the advantage of the methods of tube and cleft grafting on the method of the hole grafting, while these two methods were close to the results and did not show significant differences in the total studied parameters.

Table (1) The total results of studied parameters of the control and the grafted treatment

Grafting method / Parameter	Hole grafting	Cleft grafting	Tube grafting	Control
Percentage of grafting success (%)	++	+++	+	-
Number of seedling leaves	+	++	+++	-
Seedling length (cm)	++	+	+++	-
Wet weight of seedlings (g)	+	++	+++	-
Dry weight of seedlings (g)	+	++	+++	-

Dry matter ratio (%)	++	+++	+	-
Total/18 (100%)	9 (50%)	13 (72%)	14 (78%)	-

Oda (1995) showed that the method of tube grafting is very effective to be applied early in the plant life, which in turn eliminates the need for large rooms for acclimatization, and is essential for vegetable plants, especially tomatoes, where the success rate achieved to 85-90% and has been developed recently to be convenient for the automatic grafting (Oda, 2004).

Concern the cleft grafting method, Tremblay et al. (2003) showed that it was mainly used for *Cucurbitaceae* family plant because the diameter of seedlings stem was thicker than that of the *Solanaceae* family plant. While Rivard (2006), explained that the method of hole grafting is used to grafting tomato on eggplant, melon or cucumber, so that the scion diameter is less than the diameter of the rootstock used for grafting.

By comparing the results obtained in our experience, some of them are consistent with the results of previous studies (high success rate of tube grafting method and suitability this method for grafting of tomato seedlings, relatively low relevance of hole grafting methods to tomato scions on rootstocks from the same species). While some of the results contradict with the studies mentioned (high suitability of the method of cleft grafting for tomato scions on rootstocks of the same species).

In terms of growth strength compared to the non-grafted control, Rivard (2006) confirmed that no difference could be observed until 1-1.5 months of the transplantation process provided that the grafting was based on strong growth rootstocks, but our study did not allow us to continue the process of transplantation until the obtaining of production.

According to studies, the growth power given by the rootstock to the grafted plant is due to increased absorption of water and nutrients (Romero et al., 1997; Arao et al., 2008; Borgognone et al., 2013; Savvas et al., 2017). In this domain, the absorption of macro-elements, such as phosphorus and nitrogen, has increased in the grafted melon (Ruiz et al., 1996; Ruiz and Romero, 1999). In the eggplant grafted on tomato, a clear increase in absorption of phosphorus, calcium and sulfur was observed with all tested rootstocks, whereas in tomato, this increase was observed only in plants that were grafted on Beaufort (Leonardi and Giuffrida, 2006). Similarly, for micro-elements, especially iron, the selection of the right rootstock has played a major role in increasing the absorption of tomato plants grafted of this element (Rivaro et al., 2004). The ratio of photosynthesis (Matsuzoe et al., 1993) and the production of plant hormones (Proebsting et al. 1992) were higher in grafted tomato plants.

As previously mentioned, the tube and cleft grafting methods have been superior than hole grafting method in term of growth strength represented by length of seedlings, their wet and dry weight and the dry matter content. This can be explained by the increased ability of the grafted seedlings to absorb the water and nutrients according to the choice of the correct method of grafting.

Conclusions

At the end of this study we can conclude that all methods of grafting tested (cleft, tube and hole grafting) gave high success rates. The tube grafting method was superior to the other grafting methods in the number of leaves, leg length, wet weight and dry weight of each seedling. And also the cleft grafting method has exceeded the other grafting methods in the percentage of grafting success and percentage of dry matter. Add to that the total results of the cleft and tube grafting methods were compared in the total studied parameters. And then the method of hole grafting was delayed in all studied parameters from the other tested methods of grafting.

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