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Comparative Vegetative, Nutritional and Anatomical Study of Two Almond Varieties Grafted on Bitter Almond and Nemaguard Peach Rootstocks

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Article History

Abstract

A comparative study was conducted during the 2017/2018 and 2018/2019 growing seasons for Nonpareil and Ne Plus Ultra almond cvs grafted on Bitter almond and Nemaguard peach rootstocks at the Experimental Research Station of National Research Centre at Nubaria, El Behera governorate, Egypt. The comparison was evaluated through the grafting success percentage, vegetative growth, determination of some leaf mineral composition, as well as, the anatomical examination of scion/rootstock union zone and cross-section of leaves. The obtained results clarified that the grafting success percentage was significantly affected by the different used rootstocks. Using Bitter almond rootstock recorded the highest percentage of grafting success, leaf chlorophyll content, root length, and a number of lateral root /plant in both cultivars. Using Nemaguard peach rootstock recorded significantly the highest values of scion length, stem girth above and below union zone, number of branches and leaves/plant, leaf area as well as seedlings fresh and dry weight. Leaves of both cultivars grafted on Nemaguard peach rootstock recorded the highest concentrations of leaf mineral contents as compared to those grafted on Bitter almond rootstock. Cross-sections of the graft union zone were taken after 28 days and 6 months from grafting for anatomical analysis. The callus cells developed 28 days after grafting, but cambial cells between the rootstock and scion tissues did not occur in all scion/rootstock combinations. After 6 months of grafting, cambial cells were established, vascular differentiation was observed, regular parenchymatic tissue properties and sclerenchyma bundles were seen in the graft union. There was no problem in terms of compatibility of Nonpareil and Ne Plus Ultra on Bitter almond and Nemaguard peach rootstocks. Comparing leaf cross-sections of almond grafted on both rootstocks demonstrated that the density of mesophyll cells was affected as a consequence of the rootstock-scion interaction. Nemaguard peach rootstock can be recommended for grafting Nonpareil and Ne Plus Ultra almond cultivars under Nubaria conditions to produce vigorous trees.

Keywords: Almond; Nemaguard peach; Grafting; Rootstock; Growth; Minerals.

1. Introduction

Rootstocks contribute significantly to the production of fruit orchids, thus it is very important to use suitable rootstocks for grafting commercial cultivars [1]. The rootstocks establish the environmental suitability of the tree and its effects can be identified on the health of trees, critical stages of trees, and the sensitivity of trees in relation to insects and pathogens [2, 3]. The suitable rootstock contributes to optimal nutrient uptake, translocation and enables a reduction fertilizer application, lowering the risk of nutrient leaching and possible toxicity, without reducing fruit quality or yield [4, 5]. Almond, peaches, plums and apricot seeds are traditionally used for almond rootstock; the compatibility of almond rootstocks and almond varieties are high. Almond rootstocks are ideal for calcareous, dry, and gravelly soils and to improve the growth of the grafted varieties [6].

Bitter almond is suitable rootstock for commercial almond cultivars. However, the major defect of this rootstock is its susceptibility to nematodes and crown rot infection. Almond trees grafted on Okinawa or Nemaguard peach rootstocks were resistant to nematode, grown faster and exhibited higher yield than those grafted on almond rootstock [7]. Parvaneh, *et al.* [8], studied the influence of peach, bitter and sweet almond rootstocks on vegetative growth and nutrient absorption of 12, 15 and 18 Shahrood almond cultivars. They stated that, the growth of seedlings grafted on the bitter almond rootstock was lower than the other rootstocks while, peach rootstock gave the highest vegetative growth. Also, the rootstock had a significant impact on nutrient absorption, with peach rootstock achieving the highest level of N absorption and sweet almond rootstock for Om-El fahm almond significantly increased vigours vegetative growth and leaf nitrogen content compared with Bitter almond and Okinawa peach rootstocks.

The goal of the present research targeted to examine the impact of various rootstocks on grafting success percentage, vegetative growth and leaf mineral composition of Nonpareil and Ne Plus Ultra almond cultivars under Nubaria conditions beside anatomical investigation of graft compatibility between them.

2. Methodology

This experiment was performed during 2017/2018 and 2018/2019 seasons for evaluate the impact of different seed rootstocks; Bitter almond and Nemaguard peach on vegetative growth and leaf mineral composition of two commercial almond cultivars (scion); Nonpareil and Ne Plus Ultra at the Experimental Research Station of National Research Centre in Nubaria, El Behera governorate, Egypt. One year old seedlings rootstocks, that reached the grafting thickness, were used T-budding with chip budding method carried out in March of both seasons according to Abou Rayya, *et al.* [10]. All seedlings were grown at space 5×5 m in a sandy soil under drip irrigation system. Soil physical and chemical characteristics are present in (Table-1). The experimental design was split plot in the form of complete random block design (CRBD) with three replicate per each treatment included 5 plants for each replicate. The two cultivars were assigned as the main plot and the two rootstocks were assigned as subplot within each the main plot.

2.1. Morphological Characteristics

The following parameters were recorded assessed:

i. Successful grafting percentage (%) was assessed after two months from grafting.

ii. Length scion (cm) was measured in mid-December of both seasons by using a measuring tape.

iii. Stem girth (mm) was measured at 10 cm below and above bud-union using Vernier caliper.

iv. Number of branches/plant was counted.

v. Number of leaves/plant was counted.

vi. Leaf area (cm²) was measured using Cl-202 portable laser leaf area meter.

vii. Leaf chlorophyll content was measured in the field by using chlorophyll meter model SPAD- 502.

Survived budding seedlings were removed and washed with tap water, then subjected to the following measurements:

i. Root length (cm) was measured by using a measuring tape.

ii. Number of lateral roots/ seedling was counted.

iii. Seedling (roots and shoots) was weighted to record the seedling fresh weight (g).

iv. Roots and shoots of seedling were separated and dried at 70oC until constant weight to record the seedling dry weight (g).

2.2. Leaf mineral Contents Analysis

In the mid-August of the two seasons the leaf samples were collected from the middle part of shoots of scions and washed with tap water then dried at 70°C until a constant weight was attained to determine percentages of N, P, K, Mg, Zn, Fe, Mn and Cu (as dry weight) according to A.O.A.C [11].

2.3. Anatomical Analysis

On the 28th day and after the 6th months of grafting, samples from each combination were taken from the graft zone and were stored in FAA solution (5 ml of formalin, 5 ml of glacial acetic acid and 90 ml of ethyl alcohol 70%) until usage. For anatomical leaves sections, fresh leaves (petiole & blade) were taken from the middle part of shoots of scions and fixed in FAA solution. All cross sections were taken at 20-25 microns thick by hand, stained with safranin dye and observed under light microscope equipped with a camera [6].

2.4. Statistical Analysis

The obtained data were analyzed (ANOVA) using CoStst 6.303 statistical package and the significant differences between means were compared by LSD-test at ($p \le 0.05$).

| Sand (%) | Silt (%) | Clay (%) | Texture | OM (%) | EC dSm ¹ | pН | HCO ⁻³ (Meq./L) | CO ⁻³ (Meq./L) |
|-----------------|------------------------|-----------------|-----------|--------|---------------------|------|-------------------------------|------------------------------|
| 83.1 | 9.01 | 7.89 | Sandy | 2.84 | 0.54 | 7.88 | 1.7 | |
| CI [.] | SO_4^{-2} | Ca ⁺ | Mg^{+2} | Na^+ | \mathbf{K}^+ | Ν | Р | K |
| | Milliequivalent/ liter | | | | | | | |
| 3.2 | 0.41 | 2.3 | 2.25 | 0.3 | 0.45 | 0.99 | 0.42 | 0.54 |

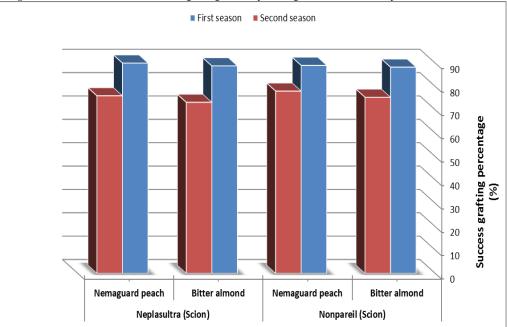
| Table-1. Soil physical | and chemical | characteristic |
|------------------------|--------------|----------------|
|------------------------|--------------|----------------|

3. Results

3.1. Morphological Characteristics

Figure-1 shows the influence of various rootstocks on grafting success percentage of almond cvs. Nonpareil and Ne Plus Ultra. It is obvious that, grafting success percentage was significantly affected by the different used rootstocks during both seasons of study. The two cultivars Nonpareil and Ne Plus Ultra grafted on the Bitter almond rootstock had higher success % (88.24 & 89% and 88.78 & 90 %) than Nemaguard peach rootstock (75.35 &78% and 73.20 & 76 %) respectively, during the two seasons.

Figure-1. Effect of various rootstocks on grafting success percentage of almond cvs. Nonpareil and Ne Plus Ultra



Tables-2, 3 and 4 show some growth parameters exhibited by Nonpareil and Ne Plus Ultra almond cultivars grafted on Bitter almond and Nemaguard peach rootstocks the grafting of these cultivars on Nemaguard peach rootstock gave higher values of scion length, stem girth above and below union zone, number of branches and leaves/plant, leaf area, weight of seedling fresh and dry comparing with grafting on Bitter almond rootstock in both seasons.

Table-2. Effect of various rootstocks on scion length, stem girth and No. of branches/plant of almond cvs. Nonpareil and Ne Plus Ultra

| Rootstocks | Scion length (cm) | | Stem girth above union zone (mm) | | Stem girth below union zone (mm) | | No. of branches/plant | |
|-----------------------|----------------------|-----------------|-------------------------------------|----------|-------------------------------------|----------|--------------------------|-----------------|
| | 1 st | 2 ^{ed} | 1 st | 2^{ed} | 1 st | 2^{ed} | 1 st | 2 ^{ed} |
| Nonpareil (Scion) | | | | | | | | |
| Bitter almond | 88 c | 91 c | 12 c | 14 bc | 13b | 14 c | 29 c | 31 c |
| Nemaguard peach | 100 b | 103 b | 13 b | 15 b | 15b | 15 b | 43 b | 45 b |
| Ne Plus Ultra (Scion) | | | | | | | | |
| Bitter almond | 82 d | 85 d | 10 d | 12 c | 10.67c | 12.00 d | 17 d | 19 d |
| Nemaguard peach | 115 a | 118 a | 17 a | 18 a | 18 a | 19.00 a | 59 a | 61 a |

Table-3. Effect of various rootstocks on No. of leaves/plant, leaf area and leaf chlorophyll content of almond cvs. Nonpareil and Ne Plus Ultra

| Rootstocks | No. of leaves/ plant | | Leaf ar | ea (cm ²) | Leaf chlorophyll content | | | |
|-----------------|-------------------------|----------|-----------------|-----------------------|-----------------------------|-----------------|--|--|
| | 1 st | 2^{ed} | 1 st | 2 ^{ed} | 1 st | 2 ^{ed} | | |
| | | | Nonpareil (| Scion) | | | | |
| Bitter almond | 273 b | 276 с | 2.37 b | 2.45 b | 50.15 a | 51.68 a | | |
| Nemaguard peach | 275 b | 280 b | 2.68 ab | 2.73 a | 47.77 a | 49.46 b | | |
| | Ne Plus Ultra (Scion) | | | | | | | |
| Bitter almond | 232 c | 235 d | 2.16 c | 2.31 c | 44.18 a | 49.95 ab | | |
| Nemaguard peach | 488 a | 491 a | 2.71 a | 2.79 a | 37.00 b | 43.47 c | | |

Table-4. Effect of various rootstocks on root length, No. of lateral root /plant, seedling fresh weight and seedling dry weight of almond cvs. Nonpareil and Ne Plus Ultra

| Rootstocks | Root Length (cm) | | No of lateral root /plant | | Seedling fresh weight (g) | | Seedling dry weight (g) | | |
|-----------------------|---------------------|----------|------------------------------|----------|------------------------------|-----------------|----------------------------|-----------------|--|
| | 1 st | 2^{ed} | 1 st | 2^{ed} | 1 st | 2 ^{ed} | 1 st | 2 ^{ed} | |
| Nonpareil (Scion) | | | | | | | | | |
| Bitter almond | 46.36 a | 50.00 a | 32.38 a | 36 a | 87.15 c | 96.07 b | 80.06 b | 91.21 a | |
| Nemaguard peach | 35.50c | 40.00 c | 21.42 c | 29 b | 98.56 a | 106.26 a | 86.00 a | 96.41 a | |
| Ne Plus Ultra (Scion) | | | | | | | | | |
| Bitter almond | 44.16a | 45.00 b | 27.55 b | 28.33 c | 78.44 d | 84.46 d | 73.55 c | 76.42 b | |
| Nemaguard peach | 38.17b | 39.33 c | 18.31 d | 21 d | 93.48 b | 95.19 c | 84.72 a | 83.55 ab | |

3.2. Leaf mineral Contents

3.2.1. Macro Elements

Table-5 shows the effect of various rootstocks on macro element contents (N, P, K and Mg) in leaves of "Nonpareil" and "Ne Plus Ultra" almond during the two seasons of study. It is obvious that, N percentage in leaves significantly increased when Nemaguard peach rootstock was used in grafting these cultivars as compared to the grafting on Bitter almond rootstock during the first season. In contrast, nitrogen leaf content was not significantly affected during the second season.

Concerning the impact of rootstocks and scion cultivar combinations on percentage of P in leaves, data in Table-5 revealed that the use of the rootstocks had no significant effect during the first and second seasons. In contrast, K percentage in leaves was significantly affected by the different rootstocks used during the two seasons. Grafting Ne Plus Ultra on Bitter almond gave the highest significant K percentage in leaves (1.55 and 1.59 %) in both seasons respectively as compared to grafting on Nemaguard peach rootstock (1.50 %) during the two seasons. Whereas, grafting Nonpareil on Nemaguard peach rootstock had higher value than grafting Ne Plus Ultra on Bitter almond (Table-5).

Mg content of the scion leaves in both cultivars was influenced by the rootstocks used. The Mg% was higher in Ne Plus Ultra almond grafted on Nemaguard peach rootstock (0.49 and 0.50 %) than Bitter almond rootstock (0.22 and 0.30 %) in the first and the second seasons respectively. Also, it has been recorded in Nonpareil on Nemaguard peach rootstock than Bitter almond rootstock in the first season. While, in the second season it was observed that rootstocks did not influence on leaf Mg content (Table-5).

| Rootstocks | Zn ppm | | Fe ppm | | Mn ppm | | Cu ppm | |
|-----------------|-----------------------|-----------------|-----------------|-----------------|-----------------|--------------|-----------------|--------------|
| | 1 st | 2 ^{ed} | 1 st | 2 ^{ed} | 1 st | $2^{\rm ed}$ | 1 st | $2^{\rm ed}$ |
| | - | | - | | | | | |
| Bitter almond | 5.65 a | 5.99 a | 83.31 ab | 83.67 c | 20.25 c | 21.00 c | 10.21 ab | 11.00 a |
| Nemaguard peach | 6.31 a | 6.52 a | 98.29 a | 97.00 a | 23.12 c | 24.33 b | 11.30 a | 12.00 a |
| | Ne Plus Ultra (Scion) | | | | | | | |
| Bitter almond | 5.94 a | 6.02 a | 67.00 b | 59.00 d | 32.11 b | 41.00 a | 8.00 c | 10.00 a |
| Nemaguard peach | 7.26 a | 7.56 a | 91.00 a | 93.00 b | 39.92 a | 42.00 a | 10.00 b | 11.00 a |

Table-5. Effect of various rootstocks on leaves macro element contents of almond cvs. Nonpareil and Ne Plus Ultra

3.2.2. Micro elements

Zn, Fe, Mn and Cu content in scion leaves of Nonpareil and Ne Plus Ultra almond were affected by using different rootstocks (Table-6). Grafting Nonpareil almond on Nemaguard peach rootstock recorded the highest values of Zn (6.31 & 6.52 ppm), Fe (98.29 & 97 ppm), Mn (23.12 & 24.33 ppm) and Cu (11.30 & 12 ppm) during both seasons respectively. The same trend was detected when used these rootstock in grafting Ne Plus Ultra almond and record Zn (7.26 & 7.56 ppm), Fe (91 & 93 ppm), Mn (39.92 & 42 ppm) and Cu (10 & 11 ppm) during both first and second seasons respectively.

| Rootstocks | Zn ppm | | Fe ppm | | Mn ppm | | Cu ppm | | |
|-----------------------|-----------------|-----------------|-----------------|--------------|-----------------|--------------|-----------------|-----------------|--|
| | 1 st | 2 ^{ed} | 1 st | $2^{\rm ed}$ | 1 st | $2^{\rm ed}$ | 1 st | 2 ^{ed} | |
| Nonpareil (Scion) | | | | | | | | | |
| Bitter almond | 5.65 a | 5.99 a | 83.31 ab | 83.67 c | 20.25 c | 21.00 c | 10.21 ab | 11.00 a | |
| Nemaguard peach | 6.31 a | 6.52 a | 98.29 a | 97.00 a | 23.12 c | 24.33 b | 11.30 a | 12.00 a | |
| Ne Plus Ultra (Scion) | | | | | | | | | |
| Bitter almond | 5.94 a | 6.02 a | 67.00 b | 59.00 d | 32.11 b | 41.00 a | 8.00 c | 10.00 a | |
| Nemaguard peach | 7.26 a | 7.56 a | 91.00 a | 93.00 b | 39.92 a | 42.00 a | 10.00 b | 11.00 a | |

Table-6. Effect of various rootstocks on leaves micro element contents of almond cvs. Nonpareil and Ne Plus Ultra

3.3. Anatomical Analysis

Figures-2 and 3 shows cross sections taken after 28 days grafting from union zone of Nonpareil and Ne Plus Ultra almond cultivars on Nemaguard peach and Bitter almond rootstocks. It is quite evident that, callus cells (Ca) developed but cambial differentiation did not begin in the sections grafted between rootstocks and scion tissues.

Figures-4 and 5 show the stem cross sections taken after 6 months grafting from the union zone of Nonpareil and Ne Plus Ultra almond cultivars grafted on Nemaguard peach and Bitter almond rootstocks. It was observed that the graft interface structure of scion cultivars and rootstocks was compatible in sections of grafts. Different tissue layers were observed in the cross section; the periderm consisting of limited number of layers followed by the cortex which often consists of several layers of chloroplast parenchymatic cells, proliferation and differentiation into new vascular cells of cambium (Cam) between the two grafted components rootstocks and scion tissues. Differentiation of wood rays to parenchymatic (Pa) and xylem (Xy) rays in the grafted interface, and complete differentiation of scion phloem (Ph). The phloem is formed from sieve tubes, companion cells, phloem parenchyma and fibers. The pith is located in the center of the stem. It is composed of parenchymatic cells and split into two zones. The outside zone cells are small in size and compact with narrow intercellular spaces, whereas the internal zone cells are large with wide intercellular spaces.

In the cross-section of scion cultivars on Nemaguard peach rootstock was detected the cortex cells which consisted of 14:16 rows of parenchyma cells and the cambium cells having 4:8 rows of meristematic cells. Whereas,

in the cross-section of scion cultivars on Bitter almond the cortex cells consisted of 12:14 rows of parenchyma cells and the cambium cells reaching 3:5 rows of meristematic cells. The amount of phloem tissues, secondary xylem rays and fibers cells of xylem were less than those of Nemaguard peach rootstock. The pith radical diameters of both rootstocks were nearly equal.

Figure-6A and B show the comparison between leaf cross sections of almond grafted on Bitter almond and Nemaguard peach rootstocks. Several indicators, such as palisade/spongy ratio, xylem/phloem ratio, vessel diameter and vessel density have been used to distinguish rootstocks [12]. The structural changes found were: (A) In the leaves of seedlings grafted on Bitter almond rootstock, the cuticle was thicker in both the lower and upper epidermis. The palisade parenchyma was thicker, more elongated and compacted, the spongy parenchyma cells were more regularly shaped and less spaced, the pith part and the xylem rays cell were smaller. (B) In the leaves of seedlings grafted on Nemaguard peach rootstock, the cuticle was thinner in both the lower and upper epidermis, also the mesophyll palisade and spongy cells were thinner, there was an increase in the number of fibers at the midrib forming a multi layered ring on the side of the phloem, and a remarkable and increase thickening of the collenchyma cell. The pith part and the xylem rays cell were bigger.

Figure-2. Cross sections in the union zone after 28 days of grafting of Nonpareil on Bitter almond rootstock (A) and on Nemaguard peach rootstock (B)

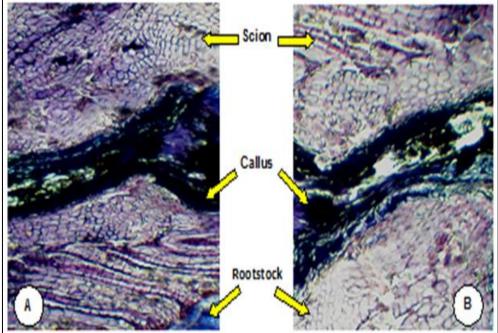


Figure-3. Cross sections in the union zone after 28 days of grafting of "Ne Plus Ultra" on Bitter almond rootstock (C) and on Nemaguard peach rootstock (D)

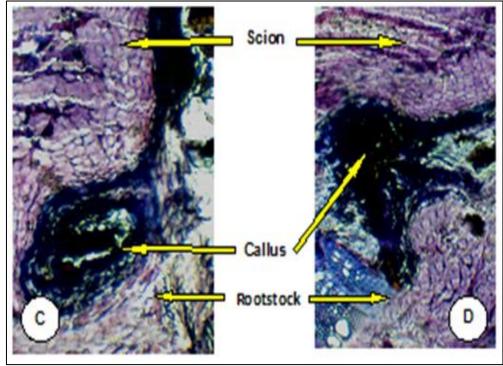


Figure-4. Cross sections taken 6 months after the grafting from union zone of "Nonpareil" on Bitter almond rootstock (A) and on Nemaguard peach rootstock (B); Callus cells (Ca), Parenchymatic cells (Pa), Cambium (Cam), Phloem (Ph), Xylem (Xy)

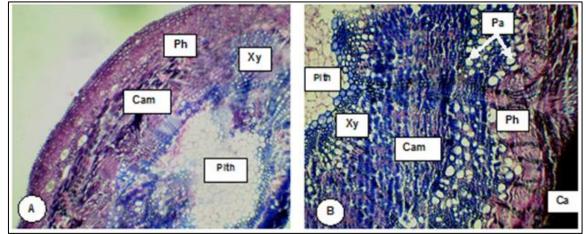


Figure-5. Cross sections taken 6 months after the grafting from union zone of "Ne Plus Ultra" on Bitter almond rootstock (C) and on Nemaguard peach rootstock (D); Callus cells (Ca), Cambium (Cam), Phloem (Ph), Xylem (Xy)

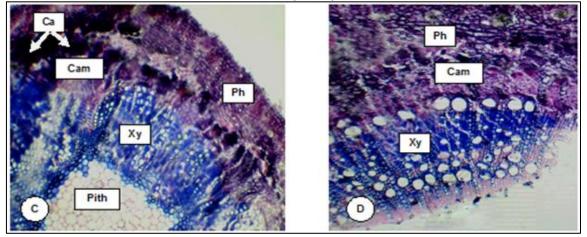
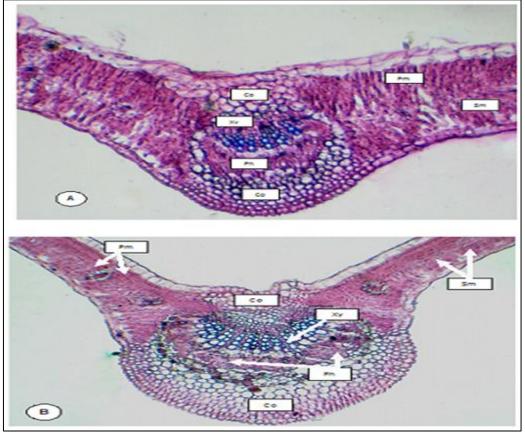


Figure-6. The comparison of leaf cross sections of almond grafted on Bitter almond rootstock (A) and on Nemaguard peach rootstock (B); Collenchyma (Co), Palisade mesophyll (Pm), Spongy mesophyll (Sm), Xylem (XY), Phloem (Ph)



4. Discussion

4.1. Grafting Success Percentage and Morphological Characteristics

The grafted fruit trees often are composed of two different parts; the rootstock and the scion. These two parts are required to be merged with each other and over time continue to grow as a one plant in grafted combinations of fruit species. Grafting success percentage was significantly affected by the different used rootstocks and no death of seedlings was observed, the experiment suggesting high graft compatibility between rootstock and scion in both cultivars. This finding is consistent with numerous studies which were conducted on scion/rootstock compatibility between some almond cultivars and almond rootstocks ¹, among almond cultivars and almond peach rootstocks [13].

Results of this study show that the rootstock type was effective on all evaluated morphological properties including; scion length, stem girth, number of branches and leaves/plant and leaf area, chlorophyll content, length and number of lateral root /plant, seedling fresh and dry weight. These results coincide with Racsko, *et al.* [2]; Parvaneh, *et al.* [8]; Eassa [9]; Sharma, *et al.* [14] who reported that using different rootstocks had a significant effect on almond growth.

In most cases, Nonpareil and Ne Plus Ultra almond seedlings grafted on Nemaguard peach rootstock had more growth than seedlings grafted on Bitter almond rootstock. These results are consistent with previous findings, that grafting Carmel and Nonpareil almond cultivars on Lovell peach rootstocks produced larger trees than the almond rootstock [7]. Also, Egea and Burgos [15] reported that the trees of Nonpareil and Merced almond cultivars were more vigorous and larger on wild peach than on Behmi and Bitter almond rootstocks. Similarly, Gall and Grasselly [16] while studying the behavior of 12 almond varieties grafted on peach, almond and peach x almond rootstocks observed that rootstocks of peach and peach x almond were superior to almond rootstock.

4.2. Leaf Mineral Contents

Leaf mineral contents of Nonpareil and Ne Plus Ultra almond was affected by the different rootstocks. Several previous studies confirmed important effects of rootstock on the mineral uptake by trees Jiménez, *et al.* [17]; Jiménez, *et al.* [18]. Also, Mayer, *et al.* [19]; Mestre, *et al.* [20] reported the impact of different *Prunus* rootstocks on nutrient status of scion. Generally, rootstocks differ in their root vigor, root cation exchange capacity, and root exudates, which can influence on leaf nutrient availability [21].

Results of this study show that leaves of both cultivars grafted on Nemaguard peach rootstock contained the highest levels of N, P, K, Mg, Zn, Fe, Mn and Cu as compared to those grafted on Bitter almond rootstock. This can be attributed to rootstock type, its role in more absorption of nutrients and genetic factors [22]. Different rootstocks genotypes can restrict nutrient translocation because of variation in xylem dimension [23], morphological and physiological aspects of root morphology, which can directly contribute to ion uptake and transport, and redistribution [24], and consequently to the final nutrient concentration for plant growth [25]. Thus, it is possible to select the rootstocks that are most efficient in capturing the mineral elements in the soil and transferring them with more effective combinations with fertilizers [26]. These results are consistent with the results of other tests on several rootstocks including almond, peach, plum and hybrids of peach x almond x plum where peach rootstock had the highest amount of elements absorption in comparison with other rootstocks [5, 8, 9, 13].

4.3. Anatomical Analysis

The connection between the rootstock and the scion anatomically occurs through the callus tissue formed by meristematic cells, which are composed of rootstock and scion, merge along a line (union zone) and the establishment of the callus bridge, after which the callus is differentiated into cambium tissues. Vascular cambium development continues and is completed across the callus bridge and is forming secondary xylem and phloem. As a result of this union, both of the rootstock and the scion side of the wood and phloem tissues allow the passage of water and plant nutrients with assimilation products from the graft union zone [27, 28].

The formation of the callus, which begins to occur two days after the grafting, takes about 2-3 weeks, and the new cambium tissue forms from the callus found between the rootstock and the scion. It is also indicated that completion of the vascular system occurs within 6-8 weeks [6].

Upon observing the anatomical cross sections between different rootstocks and almond cultivars Nonpareil and Ne Plus Ultra it was determined that callus formation between the grafting components was completed within 28 days after grafting and new transmission bundles were occurred after 6 months. An intense and regular callus formation occurred at an early stage in both rootstocks used.

Our findings are consistent with the results determined in anatomically investigated combinations of rootstockscion relationships in different species. It was stated that the vascular differentiation between the graft components in the compatible combinations was seen within the next 2-3 weeks (15-20 days) after grafting [29]. In graft combinations of *Prunus* species, it was reported that callus tissues occurred one week after grafting, cambium cells occurred after 10 days and vascular differentiation occurred after 13 days [30]. Similar results were obtained in peach and nectarine cultivars grafted on almond rootstock [31], in nectarine and peach cultivars on plum rootstock [32], in almond Ne Plus Ultra grafted on Nemaguard seedlings callus tissue filled the internal air pockets between stock and scion after 1 day for chip budding, 14 days for T-budding and 21 days for both cleft and slotted side grafting. Phloem and xylem elements were differentiated at the union zone from newly formed cambial tissues starting after 14 days and completely proliferated between the 21th and 28th day of budding and grafting respectively ¹⁰. Also, rootstock had an effect on the leaf of scion through structural changes in the number of midrib fibers on the

phloem side, and thickness of the collenchyma cells, the elongation of both the mesophyll cells (palisade and spongy), and cuticle thick Figure-6A and B.

5. Conclusions

From the illustrated data in tables and figures that included vegetative, nutritional and anatomical studies it may be recommended to use Nemaguard peach rootstock instead of Bitter almond since it recorded the highest significantly values of scion length, stem girth above and below union zone, number of branches and leaves/plant, leaf area, seedling fresh and dry weight. Also, leaves of both cultivars grafted on Nemaguard peach rootstock contained the highest amount of N, P, K, Mg, Zn, Fe, Mn and Cu compared with those grafted on Bitter almond rootstock.

Authors' Contributions

All authors contributed to the design of experiments, experimental work, preparing and reviewing the manuscript before submission.

Declaration of Competing Interest

Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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