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Pre-grafting treatments of plant fatty acid extract, seaweed (*Ascophylum nodosum* L.) and micronized calcite increase the grafting success in grapevine propagation

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Abstract

Globally increasing interest in environmentally sound viticulture in precision agriculture promoted the viticulturists to find eco-friendly treatments in both vineyards and nurseries. The present study was conducted to evaluate the possible effects of plant oil extract (as plant activator), seaweed (*Ascophylum nodosum* L.) extract, calcium oxide and iron chelate on callusing degree on graft union point and final take of 'Trakya Ilkeren' table grape cultivar grafted on 110 Richter (*Berlandieri* x *Rupestris*) rootstock. Bud break and shoot emergence commenced earlier in grafts subjected to pre-grafting immersion into iron chelate and plant oil extract during graft union room duration than those of nontreated control grafts. The greatest degree in callusing of graft union point was obtained from plant oil extract (3.8), which was closely followed by seaweed (3.7), while, on the other hand, control and immersing the graft materials into iron chelate had the lowest effect with the same value (2.2). The highest percentage of graft final take was obtained from plant oil extract (75%), followed by seaweed treatment (55%), while control and iron chelate grafts were as low as 45 and 44%, respectively. Overall findings indicated that immersing the scion canes and rootstock cuttings into plant seed oil before grafting could be recommended to increase the nursery grafting success.

Keywords: Cambium callogenesis, Final take, Grafting compatibility, Grapevine propagation, Nursey practices, Omega grafting, Precision viticulture.

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Contents

Contribution of this paper to the literature

Vticulture has been carried out in vineyards established using grape saplings grafted on different rootstocks to prevent the adverse effects of soil-borne pests such as phylloxera and nematodes. The rootstock currently used in viticulture have been bred from various North American native species. Therefore, the rootstock genotypes show a great variability in grafting affinity with cultivars. Some of them frequently failure to form adequate unity at graft point. For a sustainable viticulture, a good affinity between cultivar and rootstock has been desired to obtain healthy and abundant number of saplings. The present study revealed that plant extract effectively improved the graft success in grapevines. Therefore, the results could be useful for increasing the grapevine propagation potential with an environmentally friendly practical approach.

1. Introduction

Grape cultivars (*Vitis vinifera* L.) are important plants highly consumed for fresh table purpose as well as for the production of other foods such as raisin (dried berries), juice and wines. Various grape cultivars have long been cultivated throughout the subtropical and temperate regions around the world, especially in warm sunny climates with mild winters and dry summer periods during berry ripening. However, recently ever-increasing stress factors exacerbated by climate change, have been restricting the sustainability of grape production over the world [1]. For a precision viticulture under stress factors, grapevine cultivar and rootstock breeding studies have been promoted by different councils. General purposes in breeding of grapevine rootstocks are to provide resistance to soil born insect phylloxera (*Daktulosphaira vitifoliae* Fitch), improper soil conditions, diseases or other environmental problems [2]. North American *Vitis* species can effectively tolerate the phylloxera pest and can provide varying degrees of prevention of such soil-borne problems [3]. Therefore, the breeding and unitization of environment-adopted rootstock to cope with problematic areas should be based on many criteria such as soil features of vineyard, genetically rooting ability of the rootstock, genetic potential in vine vegetative development, usefulness in grafting and scion/rootstock graft compatibility [4-6]. Grafting practice combines two separate plant parts, a scion material (the grape cultivar selected for crop production) and a rootstock (part of the grapevine serves as its root system). Grafting the a grape cultivar onto disease-resistant rootstocks now extends to many horticultural plants [7]. However, mistakes in any process of grafting operation can inevitably cause failure in grafts success as well as serious subsequent problems difficult to compensate. Many factors play pivotal roles on the success of grafting such as exogenous synthetic hormonal applications [8] grafting technique [9] cold treatment of the cuttings before grafting, time of grafting [10] cutting shape of grafting parts [11] and environmental conditions [12]. In order to increase the graft success, synthetic chemical-based hormones such as IBA (indole butyric acid) have widely been used to provide greater callusing at graft point and faster vegetative growth [13]. However, excessive use of chemicals causes problems not only in terms of financial production cost but also in terms of the environment and public health. Therefore, the development and application of eco-friendly sustainable approaches have been gained a particular attention recently. In this context, organic plant activators such as seaweed (*Ascophylum nodosum* L. extract), fatty acid-based extracts and micronized calcium have been reported to induce vegetative development. Therefore, this study was performed to investigate pre-grafting treatments of various organic treatments on grafting success in grapevine nursery propagation.

2. Materials and Methods

The present experiment was established at the Department of Horticulture, Faculty of Agriculture, University of Selcuk in Konya Türkiye, using a glasshouse equipped with central heating unit and roof-wall ventilating systems. In dormant season hardwood canes of grape rootstock, 110 Richter with about five nodes were collected from the labelled vines as experimental rootstock material. 'Trakya Ilkeren', an early ripening hybrid hybrids of 'Alphonse Lavallee' X 'Perlette', was used as the scion cultivar.

Preparation of rootstock cuttings of 110 R and 'Trakya Ilkeren' cultivar was performed from the Grapevine Germplasm Repository of Selcuk University. Cuttings for grafting were taken from vigorous, healthy, well nourished, and mature canes generated from the previous summer season's development. The rootstock cuttings were prepared from middle portion of cane to contain 4-5 nodes. The average length and diameter cuttings prepared for grafting was 18-24 and 0.8 to 1.2cm, respectively and the cut at the base (lower end) of the cutting was performed just below the basal node while the top was cut about 3 – 4 cm above a node. The one-year-old scion ('Trakya Ilkeren') canes were taken from healthy dormant vines from vineyard. The scion cuttings of matching thickness were selected for the grafting experiments. All the rootstock and scion cuttings were treated with fungicidal solution containing carbendazim 2 g per liter by dipping them for 10 minutes and then shade dried for about 20 minutes. The scion cuttings with singly winter bud (node) were taken for grafting. Following treatments were performed just before grafting; immersing the scion and rootstock cuttings into 1) calcium oxide at 2% concentration for 2 h, 2) seaweed (*Ascophylum nodosum* L.) extract solution at 2% concentration for 2 h, 3) plant oil extract (Plant activator) at 2% concentration for 2 h, 4) iron chelate solution at 2% concentration for 2 h and 5) nontreated cuttings were used as control. After treatments, omega grafting techniques was used by using graft machine. The scion and the rootstock pieces for grafting were of similar diameter to guarantee a complete contact between the cambium of both graft pieces. Grafting operation was performed in nursery facilities, following the commercial protocol established in the nursery and described by Sabır and Ağaoğlu [8]. Briefly, one-bud (approx. 4-5 cm) hardwood cuttings of 'Trakya Ilkeren' were grafted onto approximately 25 cm de-budded canes of 110 R rootstock. The graft points were wrapped in parafilm wax applied as a dip covering the scion and graft union to prevent eternal disease inoculation and to fix graft parts tightly. Melted wax was maintained at a constant temperature of 60 to 70 °C. Waxed scion cuttings were just dipped into the cold tap water to cool. Grafting combinations were replicated 3 times, comprising a total of thirty grafted plants. Then, grafted plants were placed in a callogenesis chamber for 25 days. After callogenesis stage, the grafted plantlets were transplanted into the pots following a randomized complete block design after their uprooting for evaluation. In order to evaluate the effects of grafting methods on grafting success, the following main parameters were examined as illustrated by Celik [14] and Sabır and Ağaoğlu [8].

- Callus formation (0-4 scale) and callusing rates at graft union point after callogenesis stage: A scale ranging from 0 to 4 was used (0=no callus, 1=25%, 2=50%, 3=75% and 4=100% callus formation on graft union surface) to determine the callus formation level at graft union point.
- Rates of the grafted cuttings with different callusing levels were groped as partial callusing rate (the percentages of the grafts having the grade 1 and 2 at graft union point) and proper callusing rate (the percentages of the grafts having the grade 3 and 4 at graft union point). The percentages of the grafts having the grade 3 and 4 at graft union point was recorded as proper callusing rate (%).
- Final take rate (%) at nursery stage: Percentages of the survived young grafted vines that have an adequate vegetative development until the end of the vegetation period.
- Shoot length (cm) after nursery development: The length of the scion shoot was measured by a meter with a sensitivity of 1 mm when the vegetative development of the plant was near to cease.
- Regression analysis ($p<0.01$) was carried out to assess the correlations between the investigated parameters.

2.1. Statistical Analysis

Analysis of data variance was performed with SPSS 13.0 software program. The least significant difference (LSD) was calculated at the level of $P<0.05$.

3. Results and Discussion

Mean value of callusing degree at graft point, rates of partial or proper callusing levels obtained after the callogenesis chamber before transplanting were presented in Figure 1. The highest callusing degree was obtained from the grafts treated with plant activator (3.8), which was followed closely by seaweed (3.7) with the same significance level. Micronized calcite provided significant improvement in callusing of graft point. On the other hand, the lowest callusing degrees were determined in control (2.2) and iron chelate (2.2) treatments. These findings are in accordance with the previous reports on different cultivar7rootstock combinations [8, 15]. Anatomical union between the rootstock and the scion parts of graft is aided by a so-called tissue "callus". Wound-repair xylem is anatomically the first differentiated tissue for a proper unity of rootstock and cultivar tissues. Aside from whatever role the auxin (IAA) plays in promoting cell division at graft union point (callus formation) [16] IAA also plays an important role in xylem formation across the callus bridge between stock and scion. During this union stage, callus is formed in varying degrees under the effects of various factors such as grafting type and callusing capacities of scion and rootstock genotypes. Present findings implied that callusing capacity of the genotypes should be improved by exogenous supply of plant activators such as plant essential oil extract that contains bioactive compounds and phytohormones. The callus formation grade at graft union point is an essential factor determining the graft compatibility level between scion cultivar and rootstock [8, 14]. In addition to plant fatty acid extract, seaweed (*Ascophylum nodosum* L.) remarkably improved the callusing of grafts during callogenesis chamber.

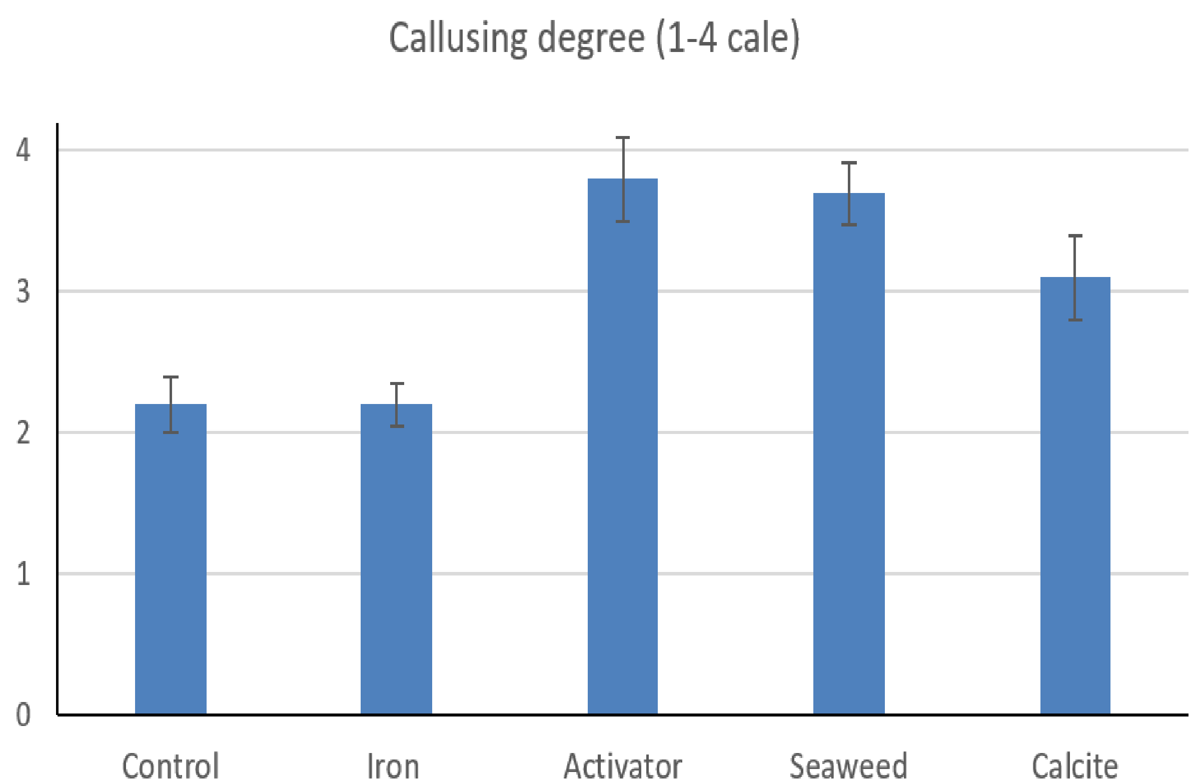

Figure 1. Changes in callusing degree (1-4 scale) at graft union point grafts in response to pre-grafting cane immersion treatments.
Note: Each column represents the mean of three replicates with ten grafts per replicate. Error bar stands for the standard deviation of that mean.

The proper callusing rate (%) of grafts was significantly improved by plant activator, seaweed and micronized calcite treatments with the percentages 83%, 80% and 73%, respectively (Figure 2). Iron chelated had no significant effect on proper callusing rate with the similar percent with control (43% and 42%, respectively). Seaweed extract has been proven to induce plant development and this promoting effect has been attributed to its betaine content [17].

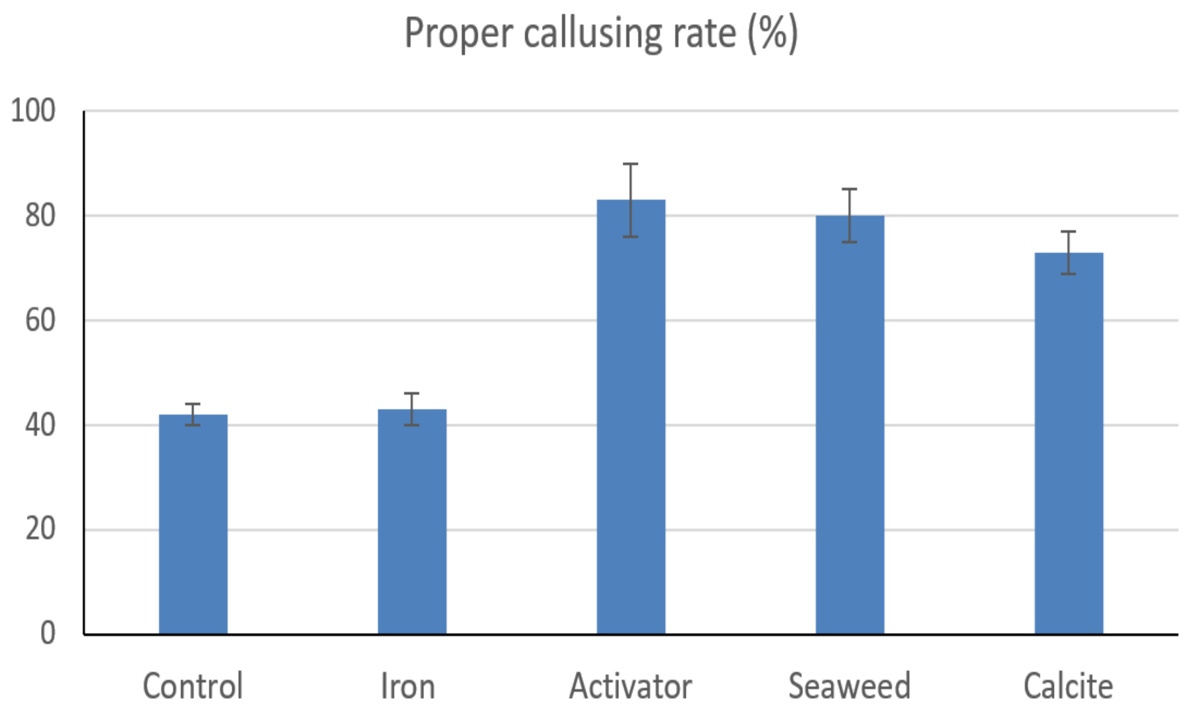

Figure 2. Changes in proper callusing rate (%) at graft union point grafts in response to pre-grafting cane immersion treatments.
Note: Each column represents the mean of three replicates with ten grafts per replicate. Error bar stands for the standard deviation of that mean.

The final take, ultimate indication of graft success, significantly differed in response to pre-grafting treatments (Figure 3). The highest final take percentage was obtained from the grafts subjected to pre-grafting soaking of plant oil extract activator (75%). This percentage is higher than those of Singh and Kaur [18] whose general rates was around 48% and lower than those of Ghojage, et al. [19] recorded around 80-81%. Such differences could be due to the genotypic variation as well as grafting condition. Cambial continuity after callusing between graft partners has also an essential role in graft survival rate [20]. Seaweed and micronized calcite treatments had also significant contributions to improvement of final take with the respective values of 55% and 51%. The lowest final take rates were determined in control (45%) and iron chelate (44%) treatments. From a practical perspective, plant activator yielded promising results as an environmentally safe and sustainable approach.

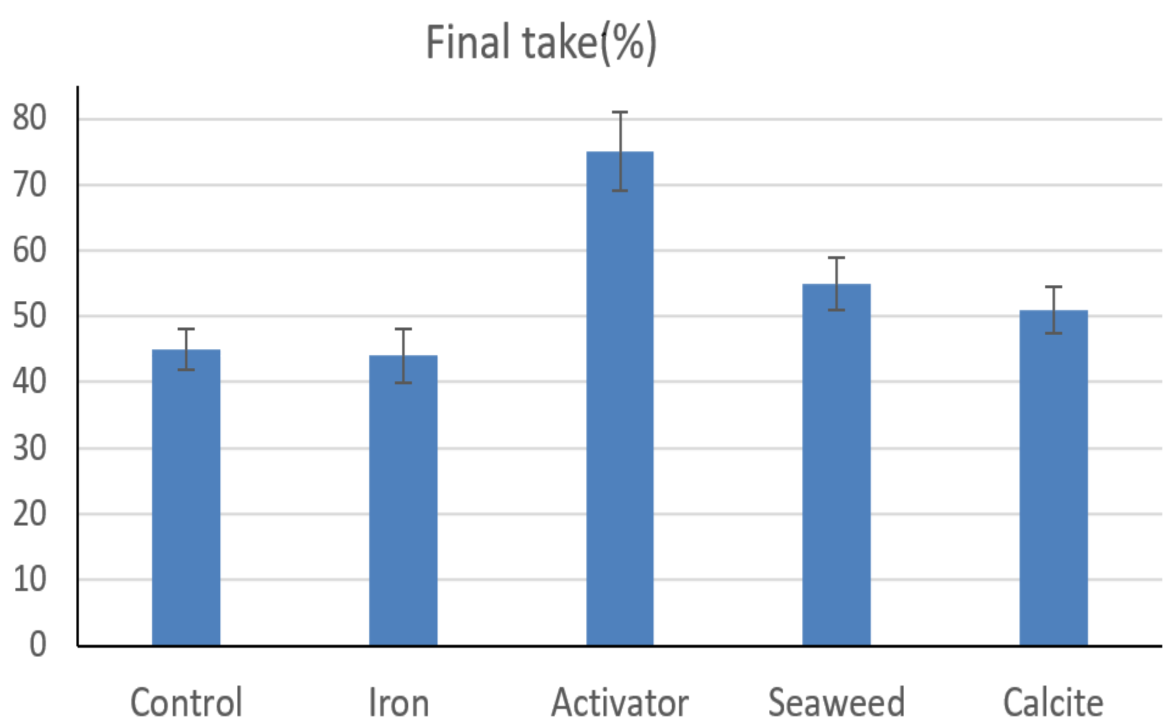

Figure 3. Changes in final take percentage (%) of grafts in response to pre-grafting cane immersion treatments.
Note: Each column represents the mean of three replicates with ten grafts per replicate. Error bar stands for the standard deviation of that mean.

Effects of various pre-grafting treatments on scion shoot length have been illustrated in Figure 4. Shoot development pattern of the scions of grafts displayed a similar pattern to that of final take. The highest shoot length was obtained from plant activator which was closely followed by seaweed. On the other hand, iron chelate and micronized calcium immersions had no significant effects on shoot development with similar values. A strong correlation between shoot growths, stock to scion ratio, and callus development indicates that growth of grafted grapevine could be different in respect to grades of callus development [14] and rootstock genotype [21]. Properly callusing at the graft union had direct influence on subsequent shoot development, an indicating of good graft compatibility [22]. Adequate shoot growth and proper lignification lengthwise the rootstock and scion trunk would determine the cold hardiness level of the saplings during the first years in vineyards for especially continental climate conditions where winter chilling injury frequently occurs.

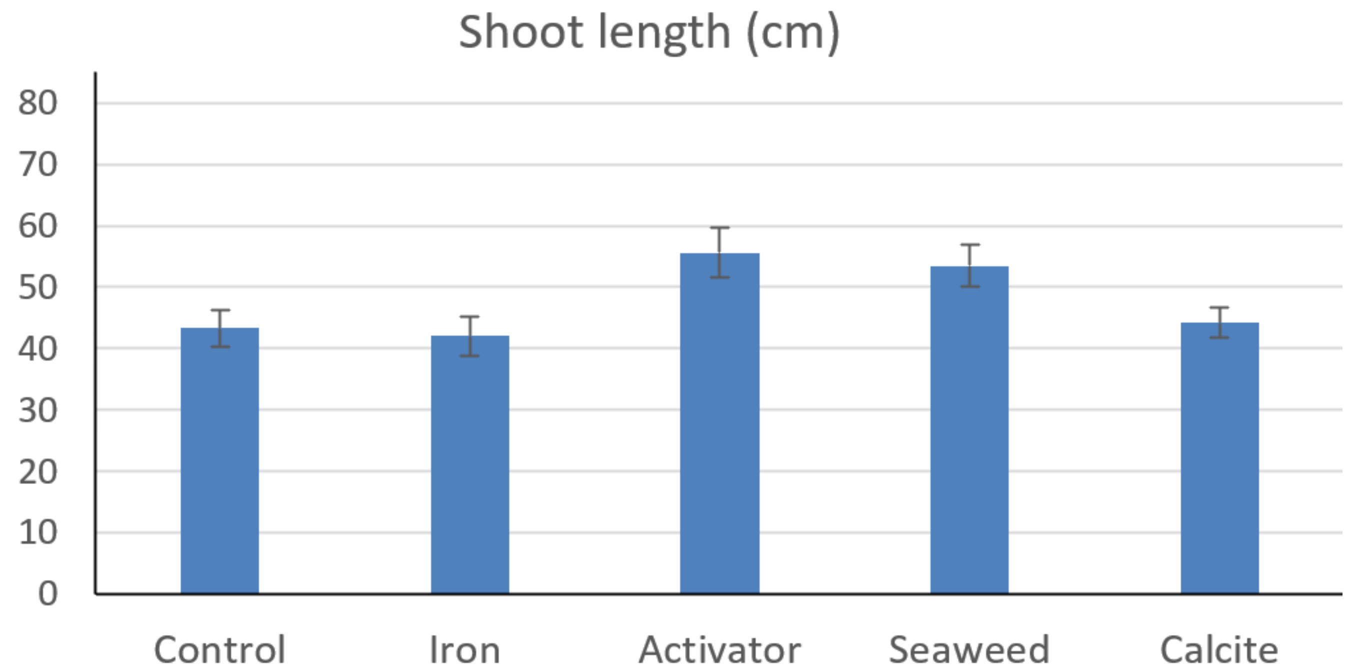

Figure 4. Changes in scion shoot length (cm) in response to pre-grafting cane immersion treatments.

Note: Each column represents the mean of three replicates with ten grafts per replicate. Error bar stands for the standard deviation of that mean.

According to regression analysis ($p<0.01$), there are significant correlations between the investigated parameters, with the greatest correlation between callusing degree (1-4 scale) and percentage of proper callusing rate (%) at graft union point (Table 1). Final take, as main indication of grafting success, showed remarkable correlations with callusing degree and percent of proper callusing rate. A proper callusing at grafts union point, a genetically controlled mechanism [23] under the effects of environmental conditions such as exogenous treatments is one of the preconditions of successful grafting [24]. There was a strong positive correlation between final take and shoot length. In arid and semiarid regions, a strong and well-lignified shoot development is desired for coping with the harsh environmental conditions [25]. Plant oil extract, serving as an activator, promoted the callusing at graft wound probably due to its phenolic acid and flavanol contents as indicated by Assunção, et al. [22].

Table 1. Correlation between the investigated parameters.

Parameters	Callusing degree	Proper callusing	Final take	Shoot length
Callusing degree	-	0.969	0.686	0.846
3.-4. degree	-	-	0.617	0.704
Final take	-	-	-	0.768

4. Conclusion

Overall investigations in the present study indicated that plant activator (plant oil extract) was the most effective pre-grafting treatment with the greatest effects on graft callusing, scion shoot growth and final take (nursery survival rate) probably due to its bioactive compound such as phenolic acids and flavanols as indicated in literature. Therefore, its application could be recommended as an environment-friendly sustainable approach in grapevine grafting. Further studies on different genotypes and various doses would yield extensive information about the use of environment-friendly substances in commercial plant propagation. In particular, graft success in the rootstocks hard to graft, such as certain *Berlandieri* hybrids (41 B), would be improved by proper use of plant activators.

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