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TECHNICAL PAPER

# Study on different grafting methods of kiwifruit 'Hayward' on the 'Matua' and 'Bruno' rootstocks

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**Abstract – Introduction.** Grafting onto kiwifruit seedlings is a common method for propagation. Grafting success rate depends on both the rootstock and grafting type. **Materials and methods.** The present study examines the effect of three grafting types on two locally prevailing rootstocks of the kiwifruit 'Hayward' in a factorial experiment based on a randomized complete block design with two factors. The first factor was the graft type at three levels (cleft, tongue and side grafting) and the second factor was rootstock at two levels ('Matua' and 'Bruno') with three replications. The measured traits included graft union percentage, scion growth, leaf area index, specific leaf area, leaf weight ratio, graft union height, number of suckers and allometric coefficient. **Results and discussion.** It was found that the highest graft union percentage (100%) was obtained under treatment cleft grafting × 'Bruno' rootstock and tongue grafting × 'Matua' rootstock, whereas the lowest graft union (66.7%) was observed in 'Matua' rootstock grafted by cleft method. Scion growth in tongue graft combination was better than other treatments. The treatment of side grafting × 'Bruno' rootstock had the highest scion growth and the treatment of cleft grafting × 'Matua' rootstock had the lowest growth. The highest number of suckers was observed under tongue grafting × 'Bruno' rootstock and the lowest number was obtained in the treatment of cleft grafting × 'Matua' rootstock. Conclusion. Cleft grafting for 'Bruno' rootstock and tongue grafting for 'Matua' rootstock with 100% success were the best and acceptable treatments.

**Keywords:** Iran / kiwi / *Actinidia deliciosa* / grafting compatibility / rootstock

**Résumé – Étude de différentes méthodes de greffage du kiwi 'Hayward' sur les porte-greffes 'Matua' et 'Bruno'. Introduction.** Le greffage est une méthode commune pour la propagation des plants de kiwi. Son taux de réussite dépend à la fois du porte-greffe et du type de greffage. **Matériel et méthodes.** La présente étude examine l'effet du greffage de la variété de kiwis 'Hayward' selon trois types de greffage sur deux porte-greffes locaux usuels dans une expérience factorielle à deux facteurs en blocs randomisés. Le premier facteur est le type de greffage à trois niveaux (en écusson, à languette ou de côté), et le deuxième facteur est le porte-greffe à deux niveaux ('Matua' ou »Bruno »), avec trois répétitions. Les caractères observés comprennent le succès du point de greffe, la croissance du scion, l'indice de surface foliaire, la surface foliaire spécifique, le rapport de poids de feuilles, la hauteur du point de greffe, le nombre de rejets et le coefficient allométrique. **Résultats et discussion.** Le meilleur succès du point de greffe (100 %) a été obtenu par greffage en fente sur 'Bruno' et le greffage à languette sur 'Matua', alors que le plus bas taux de réussite (66,7 %) a été observé sur 'Matua' en greffage en fente. La croissance du scion combinée au greffage à languette s'est montrée la meilleure de tous les traitements. Le greffage de côté sur 'Bruno' a exprimé la plus forte croissance du greffon et le greffage en fente sur 'Matua' a montré la croissance la plus faible. Le plus grand nombre de rejets a été observé avec le greffage à languette sur 'Bruno' et le nombre le plus faible a été obtenu avec le greffage en fente sur 'Matua'. **Conclusion.** Les meilleures combinaisons associent le porte-greffe 'Bruno' au greffage à languette, et le porte-greffe 'Matua' au greffage en fente.

**Mots clés :** Iran / kiwi / *Actinidia deliciosa* / compatibilité de greffage / porte-greffe

## 1 Introduction

Kiwifruit (*Actinidia deliciosa* L.) belongs to the family of Actinidiaceae. It is native to southern China and in general, to East Asia from Java to the Himalayas where it grows naturally [1]. Kiwifruit as well as avocado, blueberry and macadamia were introduced to the world in the 20$^{th}$ century [2]. 'Hayward' is a widely planted cultivar since it has big fruits weighing 100 g, delicious taste, long storage durability and high yield producing up to 50 t ha$^{-1}$. Furthermore, it is late-flowering avoiding the exposure to late spring frosts [3].

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According to FAO [4], Italy, New Zealand, Chile, Greece, France and Turkey are ranked first to sixth in terms of the cultivation area and production rate of kiwifruit. Iran is ranked seventh with the annual production of 32,000 t kiwifruit.

Grafting is a popular method of propagation fruit trees [5]. Kiwifruit can be produced via cutting, grafting onto seedlings and tissue culture. In sexual propagation, the sex of the kiwifruit tree cannot be recognized until it is flowering, *i.e.* when it is seven years old; so, it is not regarded as a useful method. The seedling will not be true to type. Therefore, asexual propagation of kiwi fruit, particularly grafting, is the best propagation method [6].

Asexual methods have been applied to many types of fruit trees. In a study on the effect of three rootstocks including 'M9', 'MM111' and 'MM106' on some traits of apple, it was determined that rootstocks significantly influenced leaf photosynthesis rate, leaf area and graft vegetative growth [7]. An evaluation of some dwarf rootstocks for selecting the best rootstock/cultivar combination of apple revealed that the rootstocks had a significant effect on increasing the trunk diameter [8]. Research revealed that different rootstocks of apple had significant impact on the yield of grafted cultivar. The rootstock is one of the factors affecting the final size of the tree [9]. In a study comparing the growth of kiwifruit 'Hayward' propagated by micropropagation, hardwood cutting and grafting, Loreti and Piccotino [12] revealed that micropropagated and grafted plants had greater vigor and resistance than plants obtained from cuttings. In a study comparing fruit yield of kiwifruits propagated by cutting and grafting methods, it was shown that micropropagated plants had the highest cumulative yield in a seven-year period [13]. Evaluation of root systems in plants propagated by grafting, cutting and tissue culture revealed that the root system of plants propagated via grafting and tissue culture was 50% better than cutting from plants [12]. It is necessary to supply the scion from parent plants with a valid family tree that are in an optimum condition in terms of health, regular productivity, yield and fruit quality. The scion should be protected carefully against bacterial and fungi infection [14].

Various factors affect the success of walnut grafting of which the important ones include temperature, environment humidity and scion [15]. Ebadi *et al.* [16] mentioned grafting time as a factor affecting grafting success. The unsuccessful buildup of a contact between vessels due to the formation of periderm in the parenchyma tissue and the abnormal placement of cambium hinders the complete union of rootstock and scion. The factors affecting the successful grafting of walnut are divided into internal (genetic) and external (environment) factors [15].

Few studies have been conducted on the effect of different rootstocks on grafting cultivars of kiwifruit. The use of wild species of Actinidia has been evaluated as rootstocks in recent years [2]. The propagation of seedling rootstocks of kiwifruit is a conventional method and kiwifruit seed germination percentage was 94% [10]. The kiwifruit can be propagated by grafting onto a seedling or other rootstock and by the use of rooted cuttings. Both seedlings and rooted cuttings are used for producing rootstocks and finally, the crop [6]. Wang *et al.* [17] compared the effect of four different species of *Actinidia,* including *A. hemsleyana*, *A. eriantha*, *A. rufa*, and *A. chinensis* as rootstocks in combination with 'Hayward' scions, with cutting propagation. They observed no significant difference in vegetative growth of grafted cultivar on different rootstocks. The lateral buds bursting time on rootstocks were almost simultaneous, while significant differences were observed in the bursting percentage of lateral buds on grafted plants. *A. hemsleyana* and *A. chinensis* had the strongest and weakest effect on lateral buds burst, respectively [17].

In a study on the different grafting methods of mango, the best time for side and cleft grafting was found to be May and July and it was reported that leaves removal of the scion 10 days before grafting resulted in the best success of grafting and plants obtained from cleft grafting had the greatest canopy [18]. In a study to determine the best time and method of mango grafting 'Langara', it was found that the best grafting time was April 4 with 100% success of grafting and the best method was cleft grafting with 85% success [19]. The purpose of the present trial was to evaluate three grafting methods on two rootstocks of kiwifruit.

## 2 Materials and methods

The present study was conducted in Talesh, Guilan province, Iran. The scions were collected from one-year-old canes and then kept in a cool and shady place for 48 h. The length of scions was 8–10 cm with two dormant buds. After transferring the potted rootstocks to the research station, grafting was carried out on February 15, 2014. The study was a factorial experiment with two factors. The first factor was grafting type at three levels including; cleft ($a_1$), tongue ($a_2$) and side ($a_3$), then the second factor was two rootstocks, *i.e.* 'Matua' seedlings ($b_1$) and 'Bruno' seedlings ($b_2$) on the basis of a randomized complete block design with three replications. Three grafting combinations were evaluated in each experimental plot. The field operations, including irrigation and fertilizations like 20 g urea, 10 g $K_2SO_4$, and 10 g triple superphosphate per 20 L water were applied at two splits in March and April. The buds of rootstocks (offshoots) started to grow in February. Then, the suckers were removed on a weekly basis.

In the cleft grafting, the limb to be grafted was split several cm with a sharp knife. Two scions were inserted in a limb, one at each end of the split. The scions had to be carefully placed so the cambium layers match. In the tongue grafting, the cuts made at the top of the rootstock had to be the same as those made at the bottom of the scion. First, a long sloping cut was made, 2.5 to 6 cm long. A second cut was made starting one- third of the distance from the tip. The stock and scion were slipped together, the tongues interlocking. For the side grafting, an oblique cut was made into the rootstock branch with a heavy knife at an angle of 20 to 30 degrees. The cut should be about 2.5 cm deep. The top of the stock branch was pulled back and the scion inserted. In all of these grafting, the scion should contain two or three buds [27].

The recorded traits included graft union percentage (%), scion growth, the height of the graft combination of ground, leaf area index (LAI), leaf weight ratio (LWR), specific leaf area (SLA), allometric coefficient (K) and number of rootstock

**Table I.** Analysis of variance of the effect of experimental factors on the measured traits (df: degree of freedom; CV: coefficient of variation; LAI: leaf area index; SLA: specific leaf area; K: allometric coefficient).

| Sources of variation | df | Means of squares | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Graft union percentage | Scion growth | Graft combination height | LAI | LWR | SLA | K | Sucker number |
| Replication | 2 | $0.001^{ns}$ | $681.76^{*}$ | $229.33^{ns}$ | $8.19^{ns}$ | $3.17^{ns}$ | $0.04^{ns}$ | $0.10^{ns}$ | $2.22^{ns}$ |
| Grating type (A) | 2 | $184.82^{ns}$ | $1720.26^{**}$ | $1079.63^{*}$ | $13.34^{ns}$ | $59.24^{ns}$ | $0.008^{**}$ | $0.86^{*}$ | $37.54^{**}$ |
| Rootstock type (B) | 1 | $246.42^{ns}$ | $234.72^{ns}$ | $1230.91^{*}$ | $2.00^{ns}$ | $101.44^{ns}$ | $0.01^{**}$ | $0.03^{ns}$ | $7.61^{*}$ |
| A × B | 2 | $800.87^{*}$ | $103.98^{ns}$ | $312.96^{ns}$ | $1.74^{ns}$ | $29.12^{ns}$ | $0.002^{**}$ | $0.50^{ns}$ | $3.12^{ns}$ |
| Error | 10 | 221.78 | 129.66 | 145.70 | 6.23 | 2.41 | 0.01 | 0.15 | 1.32 |
| CV (%) | | 16.7 | 21.76 | 20.11 | 26.87 | 22.87 | 47.96 | 28.31 | 12.26 |

$^{ns}$, *, **: non-significant, significant at $P < 0.05$ and 0.01, respectively.

suckers. LAI was calculated based on the ratio of leaf area to the ground area covered by leaves. SLA was measured on the basis of leaf area to leaf dry weight. LWR was obtained based on the ratio of leaf dry weight to total dry weight. To measure the number of offshoots (suckers), they were counted in each plot on a weekly basis.

To find root, shoot and leaves dry weight, they were oven-dried at 105 °C for 24 h. Allometric coefficient in plants shows the variation of plant growth and development of a part of plant relative to total parts or other parts. These relations are the basis for acquiring information about the status of plant growth and development. This trait is measured based on the ratio of root fresh weight to total fresh weight of plant canopy [20]. The data were statistically analyzed with the MSTATC statistical package and the means were compared with Turkey test. It should be noted that the graft union percentage was analyzed at 10% statistical level because of its importance and its means comparison carried out by Duncan Multiple Range Test.

## 3 Results and discussion

### 3.1 Graft union percentage

Results of analysis of variance (*table I*) revealed that the effect of graft type (factor A) and rootstock type (factor B) were not significant on graft union percentage, while the interaction between these factors was significant ($P < 0.10$). Means comparison of graft union percentage (*table II*) showed that the highest graft success was obtained under the treatments of cleft grafting × 'Bruno' seedlings ($a_1b_2$) and tongue grafting × 'Matua' seedlings ($a_2b_1$), whereas the lowest graft union percentage (66.7%) was observed in 'Matua' seedlings grafted with cleft method ($a_1b_1$). These findings show that the locally conventional cleft grafting method of 'Hayward' is less successful than the grafting of this cultivar via tongue method. Results indicate that grafting 'Bruno' and 'Matua' seedlings with the side method resulted in an intermediate graft union percentage (88.9%). In other words, side grafting had no statistically significant difference with other grafting methods in graft union percentage.

Khoshkhoy *et al.* [21] reported that the new cambial cells form new xylem and phloem cells, establishing connections between the vascular system of the rootstock and the scion. Therefore, one important task in grafting is to overlap the cambiums of rootstock and scion so that the callus tissues generated out of them go into each other and create a permanent bond [21]. It seems that the binding of cambium was higher in the treatments of cleft grafting × 'Bruno' rootstock and tongue grafting × 'Matua' rootstock. The problems that arose out of the interaction between rootstock and scion may not be caused by graft failure, rather by their combined genetic system, which does not adapt to inappropriate environments. Some environmental factors limiting graft union include temperature and moisture stress in graft surrounding due to scion and rootstock sensitive tissues [22].

### 3.2 Scion growth

Analysis of variance for the effect of experimental factors on the growth of scion showed that the effect of replication was significant ($P < 0.05$). It implies that the solar radiation interception and environmental factors varied among the replications. As well, the simple effect of grafting type (factor A) was significant in the growth of the scion ($P < 0.01$). It should be noted that the interaction between grafting type and rootstock type (A × B) resulted in no significant differences in this trait (*table I*). Means comparison for the simple effect of grafting type on the growth of the scion (*table II*) revealed that the treatment of side grafting ($a_3$) resulted in the highest scion growth (69.95 cm) while the treatments of cleft and tongue grafting resulted in 36.18 and 50.83 cm growth of the scion, respectively.

In citrus, when the growth of the scion is slower than rootstock, just scion determines the growth and final size of the tree [23]. The comparison of scion growth rate shows that the scion height was higher under side grafting on Bruno rootstock among treatments. It is reported that in addition to the genetic structure of the scion, various other factors are involved in the vegetative growth of scion including rootstock, soil type, and weather [24].

### 3.3 Graft union height

According to analysis of variance (*table I*), the simple effects of grafting type (A) and rootstock type (B) were significant on graft union height ($P < 0.05$) while their interaction

**Table II.** Means comparison of the effect of experimental factors on the measured traits (df: degree of freedom; LAI: leaf area index; LWR: leaf weight ration; SLA: specific leaf area; K: allometric coefficient).

| Treatments | | Means | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Graft union (%) | Scion growth (cm) | Graft combination height (cm) | LAI | LWR | SLA (cm $g^{-1}$) | K | Sucker number |
| Graft type (A) | Cleft ($a_1$) | ns | 36.18 b | 44.55 b | ns | ns | 9.04 a | 1.61 a | 7.52 b |
| | Tongue ($a_2$) | ns | 50.83 b | 67.51 a | ns | ns | 8.13 a | 1.59 a | 12.22 a |
| | Side ($a_3$) | ns | 69.95 a | 68.05 a | ns | ns | 3.20 b | 0.94 b | 8.38 b |
| Rootstock type (B) | 'Matua' ($b_1$) | ns | ns | 51.77 b | ns | ns | 4.41 b | ns | 8.72 b |
| | 'Bruno' ($b_2$) | ns | ns | 68.31 a | ns | ns | 9.16 a | ns | 10.02 a |
| AxB | $a_1b_1$ | 66.7 b | ns | ns | ns | ns | 4.84 b | ns | ns |
| | $a_1b_2$ | 100 a | ns | ns | ns | ns | 13.23 a | ns | ns |
| | $a_2b_1$ | 100 a | ns | ns | ns | ns | 5.13 b | ns | ns |
| | $a_2b_2$ | 88.9 ab | ns | ns | ns | ns | 11.13 a | ns | ns |
| | $a_3b_1$ | 88.9 ab | ns | ns | ns | ns | 3.27 b | ns | ns |
| | $a_3b_2$ | 88.9 ab | ns | ns | ns | ns | 3.12 b | ns | ns |

Same letters in a column show non-significant deference between treatments at $P < 0.05$ of Tukey test.

was not significant. Means comparison (*table II*) revealed that the graft union height was higher in the treatments of side and tongue grafting than cleft grafting. Similarly, 'Bruno' rootstock resulted in a graft union height of 68.31 cm that was superior to 'Hayward' rootstock. The rootstock and scion interaction was proven in other fruit trees. Paymaneh and Zaree [25] stated that grafted citrus have moderate growth potential and standard size on sour orange rootstock.

The ratio of the fruit quantity/tree size reflects the efficiency of assimilate partitioning between fruits and other vegetative parts of the plant. This ratio in apple trees partly depends on tree size, which depends on the effect of rootstock on tree size [26]. Size control and sometimes the shape change is one of the most dominant effects of rootstock. Rootstock has apparently changed the growth potential of the scion. If the rootstock is appropriately selected for apple trees, a complete range of tree size from very dwarf to very high can be produced by grafting a certain scion on different rootstocks [27].

### 3.4 Leaf weight ratio (LWR)

In fact, higher LWR shows a greater number of leaves on the plant. In regions with low light intensity and cloudy skies, low LWR is preferred [28]. The effect of experimental factors and their interaction were not significant on LWR (*table I*). It was found that lower leaf area in kiwifruits resulted in the loss of flower induction in the subsequent year and increased aborted flowers [2]. LWR is influenced by shading. It is lower under higher shading. In general, high LWR results in a higher net photosynthesis rate. In addition, it is estimated that a 200 $cm^2$ leaf area is needed for 100 g fresh weight apples [29].

### 3.5 Leaf area index (LAI)

It is used as a basis for the performance of tree canopy in the studies on light absorption and transpiration. The amount of radiation penetration through the canopy depends on the LAI [29]. *Table I* indicates that the effect of experimental factors and their interaction were not significant on the LAI. Buwalda and Smith [30] showed that mean leaf area of one-year-old flowering and substitute shoots of kiwifruit were 131 and 197 cm, respectively. Therefore, the competition between developing leaves and fruits for the uptake of nutrients results in the loss of leaf area on one-year-old flowering shoots [30]. When radiation is reduced (*e.g.* in cloudy days or shaded trees), the optimum leaf area is decreased. The optimum leaf area for kiwifruits in the climatic conditions of New Zealand is 3.0-3.5, which is obtained 100 days after anthesis [31]. LAI varies in the ranges of 1.5-5.0 in apple trees depending on rootstock, scion, training form, fertilization, and other agronomic operations [29].

### 3.6 Specific feaf area (SLA)

The best method to evaluate the photosynthetic efficiency of trees is to determine the increase in their dry weight per unit leaf area. The analysis of data showed that the increase in dry weight per leaf area of fruitful and fruitless apple trees was 1.07 and 0.62 kg $m^{-2}$, respectively [29]. Analysis of variance of the effect of experimental factors on the SLA (*table I*) showed that the effects of graft type (factor A) and rootstock type (factor B) and their interaction A × B were significant ($P < 0.01$). According to means comparison (*table II*), the highest SLA was observed in cleft and tongue grafting methods (9.04 and 8.13 $cm^2\ m^{-2}\ g^{-1}$), whereas side grafting had the lowest SLA. 'Bruno' rootstock had 51.89% higher SLA than 'Matua' rootstock. In addition, SLA was higher in treatment cleft grafting × 'Bruno' (13.23 $cm^2\ m^{-2}\ g^{-1}$) and tongue grafting × 'Bruno' (11.13 $cm^2\ m^{-2}\ g^{-1}$) than other treatments, while it was the lowest in the treatment of side grafting × 'Bruno' (3.12 $cm^2\ m^{-2}\ g^{-1}$). Smith *et al.* [32] reported that the increase in SLA after a critical level not only had no positive effect on the quantity and quality of flowering and crop, but also resulted in adverse effect of shading of canopy. A review

of literature [2] shows that in apple trees, 106–109 $cm^2$ leaf area per spur is needed for the maximum flowering. This ratio is reported as to be 225 $cm^2$ for kiwifruit [2].

### 3.7 Allometric coefficient (K)

Allometric coefficient in interspecies comparison is a foundation for being paid information about the growth and development of plants. Various studies show that the plants' ability in adapting to the environment affects allometric relations [20]. Analysis of variance for the effect of experimental factors on allometric coefficient (*table I*) indicated that the simple effect of grafting type (factor A) was significant on this trait ($P < 0.01$), whereas the simple effect of rootstock (B) and the interaction grafting and rootstock type was not significant.

Means comparison of grafting type on allometric coefficient (*table II*) showed that the highest allometric coefficient of the grafted plants was obtained in cleft ($a_1$) and tongue ($a_2$) grafting (1.60 and 1.59, respectively) while side grafting ($a_3$) had the lowest one (0.94). The comparison of the canopy of 8-year-old trees grown from the grafting of cv. Cox on '$M_9$' rootstock showed that the volume of canopy when the grafting was carried out at the height of 75 cm was 59% as great as that when it was done at the height of 15 cm [26]. In addition, Aslani *et al.* [26] stated that '$M_9$' as an inter-stock had a greater influence on root growth than on shoot growth, so that root growth is reduced to 50%. The canopy of the grafted trees also affects the root number and branching. If the canopy has numerous main branches, the root will have many branches as well because each root usually has the direct responsibility for feeding a branch [5].

### 3.8 Number of suckers

According to analysis of variance for the effect of studied factors (*table I*), the simple effect of grafting type and rootstock type were significant on the number of suckers. However, the interaction of grafting and rootstock type was not significant on the number of suckers in kiwifruit. Means comparison for the number of suckers of kiwifruit (*table II*) revealed that the highest number of suckers (12.22) was produced with tongue grafting ($a_2$) whereas the lowest number (7.52) was observed in cleft grafting ($a_1$). In addition, 'Bruno' rootstock ($b_2$) had a greater number of suckers than 'Hayward' rootstock ($b_1$) (10.02 and 8.72, respectively). It was found that number of suckers was 48.5% higher in tongue grafting than in cleft grafting. Hartmann *et al.* [27] reported that *Prunus spinosa* is the most dwarf rootstock for plum but it has many problems in excessive suckering. Therefore, it should be used as a hybridizing parent in breeding programs. Qasemi [33] grafted local varieties of pear on four quince (*Cydonia oblonga* Mill.) rootstocks and studied the compatibility of rootstock and scion and the rate of suckering. He found that the rootstocks of quince affected the vegetative growth of the trees.

## 4 Conclusion

In conclusion, cleft grafting for 'Bruno' rootstock and tongue grafting for 'Matua' rootstock with 100% success were the best and most acceptable practices. However, the initial growth of side-grafted plants was better than other methods. Our results revealed that 'Bruno' had a stronger tendency to produce suckers than 'Matua'.

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