'Z1 VITROPLANT' - VALUABLE ROOTSTOCK FOR KIWIFRUIT CULTIVARS - GRAFTING RESULTS

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Abstract

Grafting of kiwifruit cultivars is more and more a necessity due to some soil born problems as high calcium and pH, plants die-off or "Kiwi Moria" etc. A solution can be the use of 'Z1 Vitroplant' rootstock (A. deliciosa x A. arguta hybrid obtained at Vitroplant Italia) that shows good resistance to cold, ferric chlorosis, low permeable soil and water scarcity. 'Hayward' and 'Bruno' cultivars, and some new valuable hybrid genotypes, obtained through intra and interspecific crosses, were used for grafting. The 'Z1' rootstock was planted in 10 liters pots and grafted in April, in a cold greenhouse, where the temperature varied between $20-24^{\circ}$ C. Scions were taken from mother plants in January and preserved in dormancy at $2-3^{\circ}$ C. Whip and tongue grafting was used. Grafting success rate, rootstock, woody scion and main scion shoot diameters, total scion shoots length, total number and average shoots length, were analysed at 4 months after grafting. The results showed significant differences between cultivars for most of the analysed parameters.

Key words: Actinidia sp., growth, propagation, whip and tongue.

INTRODUCTION

Kiwifruit (*Actinidia* Lindl.) is a recently developed horticultural crop, with a very short history of cultivation (Sui et al., 2013). According to the latest revision, *Actinidia* genus belongs to the *Actinidiaceae* family and has over 75 species and about 125 known taxa worldwide (Huang & Ferguson, 2007).

Even the centre of origin is China, most of the *Actinidia* species are widely distributed in Asia (Huang & Ferguson, 2007). As Cui (1993) and Stirk (2005) mentioned, *Actinidia* species are found in different climates and geographical environments, from India to Japan and from Siberia to Indonesia.

Huang (2016) and Zhang et al. (2010) reported that the most common kiwifruit species are *A. deliciosa* and *A. chinensis* and the current commercial cultivation is almost entirely based on these ones. Lesser extent, *A. arguta* commercial potential started to be recognised, in colder regions, in the early 20th century (Ferguson & Huang, 2007).

Kiwi is a very appreciated fruit due to its nutritional properties, high vitamin C content, as well as its taste and flavour (Biao et al., 2018; Yang et al., 2010; Young et al., 1995). Also kiwifruit is a rich source of vitamin E, vitamin K, vitamin B complex, carotenoids, choline, minerals (Na, K, Ca, Mg, Mn, Fe, Cu, Zn), dietary fiber etc. (Çeliket et al., 2006a; Ferguson, 1999; Jesion et al., 2013; Kim et al., 2010; Mohammed et al., 2017).

The *Actinidiaceae* is a family of woody, deciduous and perennial vigorous vines (Ferguson, 1984; Stirk, 2005).

As most of the fruit trees, kiwifruit plant can be propagated by seedlings or by asexual methods, such as grafting, semi-hardwood or hard wood cuttings, and also tissue culture (Kumar & Sharma, 2002; Lawes, 1992; Peticilă et al., 2012a; Sale, 1985; Stănică et al., 2003a).

Because of its dioeciously nature, propagation from seed of kiwifruit for commercial plantations, are not recommended, the plant sex being unknown until flowers are produced (Sedaghathoor & Noie, 2016). Also, the seedlings fruiting starts later compared to the vegetative multiplied plants.

Sexual propagation is mostly used to produce rootstock seedlings for grafting (Irshad et al., 2014). According with Hartmann et al. (2011), Stănică et al. (1995), Stănică (2004a) and Tanimoto (1994), the common methods of kiwifruit propagation are grafting, cuttings and micropropagation.

Nevertheless, previous researches have shown that kiwifruit cuttings are characterized by a very low intrinsic rooting ability. This is why, particular techniques as, bench heating, temperature control, fog or mist, as well as rhyzogenetic substance treatments are always required in order to obtain satisfactory results (Alam et al., 2007; Babar et al., 2018; Biasi et al., 1990; Dumitrașcu et al., 2003; Ono et al., 2000; Peticilă et al., 2015; Peticilă et al., 2016; Stănică et al., 2003b; Zenginbal & Özcan, 2014). Root formation was strongly influenced by species, variety and rooting time (Kumar & Sharma, 2002).

Regarding *in vitro* kiwifruit propagation, according with Akbaş et al. (2007), Famiani et al. (1997), Ferradini et al. (1996), Irshad et al. (2014), Kumar & Sharma (2002), Ono et al. (2003), Peticilă et al. (2012b), Stănică & Armeanu (2004b) and Stănică et al. (2005), culture medium, genotypes and time of inoculation are very important for propagation rate.

Grafting is a vegetative method of propagation, often used in horticulture (Stănică et al., 2003a) with major applicability: introduction of cultivars with high biological characteristics, resistant or tolerant to pests and diseases, tolerant to abiotic stress factors, reduction of soil borne problems, improvement of water and nutrients absorption etc. (Çürük et al., 2009; Doltu et al., 2017; King et al., 2010; Lee, 1994; Lee et al., 2010; Rivero et al., 2003; Webster, 1995).

According to Hartmann & Kester (1975), the origin of grafting can be traced back to ancient times, the Chinese being familiar with the art of trees grafting at least as early as 1000 B.C. Throughout the time a large number of scientific research are reported in the literature concerning grafting in different horticultural crops: vegetables (Doltu & Bogoescu, 2014; Doltu et al., 2017; Ergun & Aktas, 2018; Lee, 1994; Oda, 1995; Rouphael et al., 2010; Sakata et al., 2007), flowers (Fang et al., 2009; Weinard & Dorner, 1927; Zhang et al., 2013), ornamental plants (Hinesley & Frampton, 2002; Jayawickrama et al., 1991; Melnyk &

Meyerowitz, 2015; Roberto & Colombo, 2020; Tarroux & DesRochers, 2011), wine grapes (Cimpoi et al., 2020), fruit trees (Asănică & Tudor, 2011; Asănică et al., 2013; Bărăscu et al., 2018; Hoza et al., 2020; Stănică, 2019; Tabacu et al., 2020; Vercammen et al., 2007).

The most common types of grafting often used in fruit nurseries and orchards are represented in Figure 1: a) twin cleft whip grafting or tongue grafting; b) bud grafting (chip-budding or T-budding); c) notch grafting; d) cleft grafting.

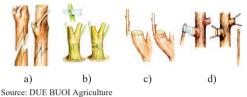


Figure 1. Common grafting types in fruit nurseries and orchards

For kiwifruit, the main used grafting methods are top grafting (tongue and cleft grafting), chip-budding or T-budding, and also side grafting (Luh & Wang, 1984; Sedaghathoor & Noie, 2016; Zenginbal et al., 2006a; Zenginbal et al., 2006b).

Top grafting is one of techniques frequently used in cultivars replacement and improvement (Huang, 2006; Liang et al., 2011; Liu & Wang, 2006).

Important contributions to the study of the *Actinidia* graft budding were made also by Çelik et al. (2006b), Gustafson & Morrissey (2003) and Zenginbal et al. (2006b).

Corresponding to several study, whip and tongue grafting is a suitable method for asexual propagation of kiwifruit (Mohammadi & Abdi, 1993; Pandey, 2019; Zenginbal et al., 2006a).

The grafting success can be affected by several factors such as temperature, humidity, scion variety, rootstock, grafting time, wrapping materials, grafting methods, pests and diseases etc. (Hamdi et al., 2007; Pandey et al., 2019; Tanimoto, 1994).

Few studies have been conducted regarding the behaviour of different rootstocks grafted with kiwifruit cultivars (Sedaghathoor & Noie, 2016; Zuccherelli, 1979). In the recent years, the use of wild species of *Actinidia* as rootstocks has been also evaluated (Liang et al., 2011; Sedaghathoor & Noie, 2016). Kiwifruit rootstocks can be propagated by seedling or rooted cuttings (Anderson & Lawes, 1980; Mohammadi & Abdi Senehkouhi, 1993; Pandey et al., 2019; Sedaghathoor & Noie, 2016; Zenginbal et al., 2006b).

Özcan (2000) and Sedaghathoor & Noie (2016) mentioned in their research that seedlings have high vigor and long roots than cuttings.

Different species of *Actinidia*, including *A. chrysantha*, *A. eriantha*, *A. globosa*, *A. hemsleyana*, *A. kolomikta*, *A. macrosperma*, *A. polygama*, *A. rufa* were used as rootstocks in combination with various scions cultivars (Clearwater et al., 2004, 2006, 2007; Liang et al., 2011; Wang et al., 1994). Also, some cultivars of *A. arguta*, *A. chinensis* and *A. deliciosa* ('Matua', 'Bruno', 'Hayward') obtained by seedlings or rooted cutting, were used as rootstock in grafting propagation (Çelik et al., 2006a; Sedaghathoor & Noie, 2016; Zenginbal et al., 2006b).

In Romania, kiwifruit research and culture started in 1993 (Peticilă et al., 2002; Stănică & Cepoiu, 1996; Stănică, 2009; Zuccherelli, 1994). The most important studies were conducted in a common Romanian-Italian kiwifruit breeding program, initiated at the Faculty of Horticulture within the University of Agronomic Sciences and Veterinary Medicine of Bucharest (Stănică & Zuccherelli, 2007; Stănică & Zuccherelli, 2009).

Taking into consideration that grafting of kiwifruit cultivars is more and more a necessity due to some soil born problems as high calcium and pH, plants die-off or "Kiwi Moria" etc., this study can provide some solutions by using a resistant rootstock as 'Z1' showed to be.

MATERIALS AND METHODS

Experimental Site

The study was conducted in 2020, at the Faculty of Horticulture cold greenhouse, within the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania.

According to Asănică & Tudor (2011), the climate in the experimental area is typically temperate-continental, with cold winter and warm summer. Springs registered significant variations between day and night amplitude, and autumns distinguished by moderate thermal and slow transition to winter (Tudor et al., 2014).

Plant material

'Hayward' and 'Bruno' cultivars and eight Romanian intra and interspecific *Actinidia* hybrids, were grafted. The scions trial used for this study is presented in Table 1.

Scions were taken in early January from vigorous productive plants, grown in the Experimental Field of the Faculty of Horticulture, Bucharest. The shoots were preserved in dormancy until the grafting moment, at 2-3°C, wrapped in plastic film.

	Table 1. Scions	s cultivars	and hybrids	description
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Variety/Hybrid		Species		
Hayward (♀)	_	A. deliciosa		
Bruno (♀)		A. deliciosa		
R0P9 (්)		A. chinensis intraspecific hybrid		
R0P10 (♀)		A. chinensis intraspecific hybrid		
R0P13 (♀)		A. deliciosa x A. chinensis interspecific hybrid		
R1P1 (♀)	۲	A. deliciosa x A. chinensis interspecific hybrid		
R1P8 (♀)		A. deliciosa x A. chinensis interspecific hybrid		
R1P9 (♀)		A. deliciosa x A. chinensis interspecific hybrid		
R1P12 (♀)		A. deliciosa x A. chinensis interspecific hybrid		
R9P20 (Vip Red) (♀)		A. arguta		

One-year-old 'Z1 Vitroplant' rootstocks, in vitro propagated, were planted in 10 liters pots, and grafted in April, in a cold greenhouse, where the temperature varied between 20-24°C. 'Z1 Vitroplant' (Vip Zedone ®) rootstock is a hybrid of A. deliciosa ('P1') and A. arguta ('Gemma'), obtained at Vitroplant, that shows good resistance to cold, ferric chlorosis, low permeable soil and water scarcity (Zuccherelli, 1994). The plants have medium vegetative vigor and good affinity with the Actinidia and Α. chinensis deliciosa genotypes. According Vitroplant Italia, 'Z1 Vitroplant' is

resistant to PSA and is currently in an advanced experimentation for the evaluation of its resistance to the so-called "Kiwi die-off" ("Kiwi Moria"), with satisfactory results.

Grafting

Twenty-four hours before grafting, the scions were placed with the base in water for hydration. The rootstock plants were also watered. Whip and tongue grafting was applied (Figure 2) and Flexiband was used as wrapping material. To reduce water loss and oxidation, Arborinn special wax was applied.

Cultural operations such as irrigation, weeding and removal of suckers below the grafting point followed at regular intervals. Four months after grafting (middle August), the anticipated shoots on the main scion shoot, were pinched at 20-25 cm. Flexiband has been removed to avoid strangulation of grafting point (generally, it is naturally degraded by UV in open field).



Figure 2. Kiwifruit whip and tongue grafting phases

Data collection

Data on sprouting were recorded after bud burst, while the grafting success percentage and the other observations and measured traits were recorded four months after grafting.

The number of days from grafting to the first bud burst was recorded. Starting with the 20th day after grafting, the percentage of the sprouting plants was calculated, every 10 days. Grafting success rate was calculated following formula and was express by percentage:

Graft success rate = $\frac{\text{Number of sprouted graft}}{\text{Total grafted plants}} \times 100$

Average diameters of rootstock, woody scion and main scion shoot were measured with an electronic calliper and expressed in millimetres (mm).

Main scion was measured in centimetres and the average length was calculated. The length and the total number of anticipated shoots were also recorded.

Statistical analysis

Data statistical analyses were performed with Excel (MS Office).

RESULTS AND DISCUSSIONS

Callus along the union point began to occur in about two weeks after grafting.

Scions sprouting percentage at different days after grafting

The dynamic of plants sprouting percentage between 20 and 60 days after grafting, recorded every 10 days, is presented in Figure 3.

At the 20th day after grafting, all genotypes had sprouted plants.

After 30 days, most of the scion cultivars recorded over 50.0% sprouted plants, excepting 'Hayward' (30.0%) and R1P12 (40.0%).

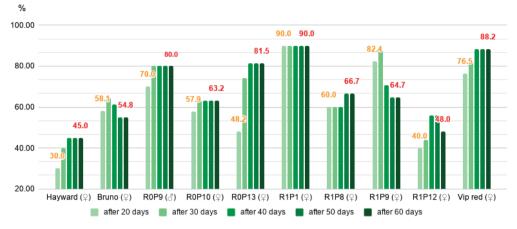


Figure 3. Dynamic of kiwi plants sprouting (%) after grafting



Figure 4. Scion shoot growth of R1P1 genotype (30 days after grafting)

At 60 days after grafting, the minimum percentage of scion sprouting was recorded at 'Hayward' (45%) and the maximum, at R1P1 (90%).

The sprouting percentage increased constantly till the 60^{th} day after grafting, for almost cultivars. 'Bruno' and R1P9, registered a decreasing tendency from 58.1% to 54.8%, and from 82.4% to 64.7% respectively due to some plants lost.

R1P1 registered the highest sprouting percentage (90.0%) after 20 days.

According Bose et al. (2019), the variation of cultivars sprouting is due to genetic differences in translocation of food reserves and change in the cambial activity.



Figure 5. Grafting success rate (%)

Grafting success rate

The observation regarding kiwifruit grafting success rate on 'Z1' rootstock, are represented in Figure 5. The highest percentage of survived grafts was obtained at R1P1 genotype (90.00%), followed by Vip Red (88.24%), R0P13 (81.48%) and R0P9 (80%), while the lowest, was registered at Hayward (45.00%). Only Hayward (45.00%) and R1P12 (48.00%)

registered lower values of grafting success rate than 50%. The effect of different scion variety on grafting success rate was significantly different at four months after grafting. Hartmann et al. (2007) reported that genetic

factors had a significant effect on grafting success. The ability of two kinds of plant to form a successful graft union is largely based on their natural relationship (Sharma, 2002).



Figure 6. Grafting point union and main scion shoot details, after 4 months, for 'Bruno' and R1P12

Rootstock and woody scion diameters

Rootstock and woody scion diameters at the grafting point have quite similar values (Figure 7). The differences between rootstock and scion shoots diameters varied from 0.13 mm at 'Bruno' to 1.52 mm at R1P1.

Only in the case of the R1P12 genotype, the average diameter of the scions was thicker than the rootstock one.

Plants grafted vigour and total vegetative growth

In Figure 8, can be observed how the grafted plants on 'Z1 Vitroplant' looked at 90 days after grafting. Main scion shoot diameter (mm) and length (cm), total number and length of primary laterals shoots, total number and length of secondary laterals shoots, were presented in Table 2.

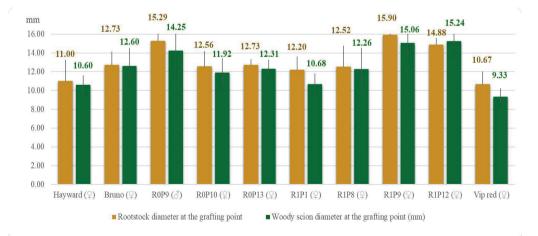


Figure 7. Rootstock and woody scion shoot diameters at the grafting point



Figure 8. Grafted plants on 'Z1 Vitroplant' at 90 days after grafting

Main scion shoot diameter varied from 6.15 mm for 'Vip Red' to over 10 mm at R0P13, R1P9, R0P9 and R1P12.

R0P9 recorded the highest value of main scion average shoot length - 387.20 cm. The lowest value was registered at 'Hayward' 144.83 cm. High values were measured also at R0P13 (275.40 cm), Bruno (253.43 cm), R1P12 (246.50 cm) and R1P1 (231.80 cm). All genotypes presented anticipated lateral shoots. The most vigorous genotypes, with the highest number of anticipated shoots are 'Vip Red' (9.50), R0P13 (5.40), 'Bruno' (5.14), R1P9 (5.00) and R0P10 (5.00). The lowest number was registered at 'Hayward' (2.17).

Few plants from R1P12, R1P8, R1P1 and R0P10 genotypes, formed second anticipated lateral shoots. The longest values of the second anticipated shoots were registrated at R0P10 (60.00 cm) and R1P8 (47.50 cm).

Cultivar/Hybrid	Main scion shoot diameter (mm)	Main scion shoot length (cm)	Total anticipated shoots (no)	Anticipated lateral shoots length (cm)	Total second anticipated shoots (no)	Second anticipated shoots length (cm)
Hayward ($\stackrel{\bigcirc}{\downarrow}$)	$8.65{\pm}0.66^{*}$	144.83	2.17	48.46	0	-
Bruno (♀)	9.34±0.65*	253.43	5.14	59.69	0	-
R0P9 (්)	10.62 ±0.88*	387.20	3.33	67.00	0	-
R0P10 (♀)	9.44±2.71*	185.40	5.00	55.20	0.20	60.00
R0P13 (♀)	10.97 ±1.81*	275.40	5.40	50.19	0	-
R1P1 (♀)	9.66±0.95*	231.80	3.00	60.82	0.20	38.00
R1P8 (♀)	9.25±1.65*	198.50	3.44	79.39	0.33	47.50
R1P9 (♀)	10.72 ±1.21*	228.20	5.00	33.16	0	-
R1P12 (♀)	10.41 ±1.15*	246.50	3.50	71.81	0.67	23.50
Vip Red (♀)	6.15±0.35*	222.23	9.50	45.47	0	-

Table 2. Kiwi plants vigour and total vegetative growth

* Standard deviation

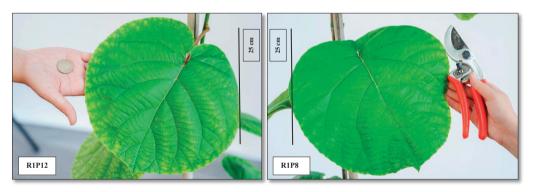


Figure 9. R1P12 and R1R8 kiwifruit hybrids leaf extension at 90 days after grafting

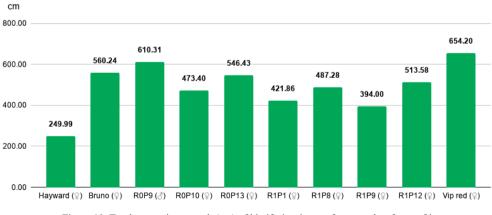


Figure 10. Total vegetative growth (cm) of kiwifruit scions at four months after grafting



Figure 11. R1P12 main scion and anticipated laterals shoots (90 days after grafting)

The average of total vegetative growth, calculated at four months after grafting, are represented by the average length of main scion shoot and the sum of the average length of anticipated shoots (Figure 10).

'Vip Red' and R0P9 registered the highest value of total vegetative growth (654.20 cm and 610.31 cm, respectively).

'Vip Red' formed also the biggest number of anticipated shoots.

The lowest total vegetative growth was registered at 'Hayward' (249.99 cm).

CONCLUSIONS

The results showed significant differences between cultivars and genotypes for most of the analysed parameters. Based on the observations and measurements we can conclude that, most of the chosen kiwifruit genotypes grafted on 'Z1 Vitroplant' showed good results. R1P1 registered the highest grafting success rate (90%) and showed good results regarding the growth vigour. The highest values of the total vegetative growth were registered by 'Vip Red' (654.20 cm) and R0P9 (610.31 cm).

⁶Z1 Vitroplant' rootstock showed good grafting compatibility with all tested kiwifruit cultivars and hybrid genotypes. Grafted plants will be tested in the experimental orchard for productivity and other field resistances.

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