Seedling Production of Kiwifruit Using Hardwood and Semi-hardwood Cuttings in Greenhouses Under Controlled Atmosphere Conditions

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Abstract

While the area under cultivation and kiwifruit production in the world is increasing year by year, a big issue is the seedling production. The paper presents the influence of hardwood and semi-hardwood cuttings of kiwifruit on seedling production. The study was conducted during two consecutive years, 2011-2013, in a controlled atmosphere greenhouse in Lezha, using three planting periods each year: 5th March (3 variants using hardwood cuttings with different cutting and storage period), 5th September (1 variant using semi-hardwood cuttings) and 5th October (1 variant using semi-hardwood cuttings). A randomized complete block design (RCBD) with 5 variants and three replications for variant, with a plot size of 100 cuttings, was used. Vegetative cuttings were treated with eight different rates of indole-3-butyric-acid (IBA) crystalline concentration solutions (0-5000 ppm) and IBA talc 2000 ppm, and were placed for rooting on a perlite-peat mix (60:40) supporter, under controlled environmental conditions (air temperature 22-26^{o}C, basal perlite-peat mix temperature 17-18^{o}C, and 90-95% RH). Obtained results showed that hardwood cuttings placed for rooting in 5th March rooted better than semi-hardwood cuttings planted in 5th September and 5th October, giving the highest rooting percentage (76-92%). The highest rooting percentage (92%) and the highest quality seedlings (76.8% with root length >15 cm and 71.1% with vine length >20 cm) were achieved using hardwood cuttings cutted and placed immediately for rooting in 5th March, treated with IBA 3000 ppm. Differences between variants and treatments were testified using LSD test (0.05).

Keywords: hardwood cuttings, IBA talc, indole-3-butyric-acid, kiwifruit, rooting percentage, seedling production, and semi-hardwood cuttings.

INTRODUCTION

Kiwifruit (*Actinidia deliciosa* C. F. Liang & A. R. Ferguson) is very interesting and a very important fruit tree in the world's horticultural history, in Albania as well. Over 70% of kiwi production is in Italy, New Zealand, and Chile. Italy produces roughly 10% more kiwifruit than New Zealand, and Chile produces 40% less (FAOSTAT, 2011; Bano and Scrimgeour, 2011). With these three main production centers, kiwifruit is produced for worldwide consumption roughly all year long. According to

United Nation Food and Agriculture Organization (FAO, 2012; Wilkinson, 2008), world kiwifruit production in 2012 was 1412351 tonnes and ten top kiwifruit-producing countries were Italy (384344 tonnes), New Zealand (376400 tonnes), Chile (240000 tonnes), Greece (161400 tonnes), France (65253 tonnes), Turkey (36781 tonnes), Iran (32000 tonnes), Japan (28000 tonnes), US (26853 tonnes), and Portugal (25000 tonnes). According to Huang and Ferguson (2003; 2001), within a few years, China will be producing much more kiwifruit from commercial orchards than any other country and this is likely to have a significant impact on international trade in kiwifruit.

Kiwifruit, in the Albanian market, was present after 1990, mainly imported from Greece and Italy. Kiwifruits domestic cultivation started in 1994, in Balldre, Lezha district, with seedlings comming from Montenegro. After 2002, with the support of World Bank in Albania, there were planted some other new kwifruit orchards in Velipoja (Shkodër), Divjaka (Lushnjë), Elbasan, Llakatund (Vlora), Seman (Fier), etc, showing a very high fruit quantity and quality, under plain Mediterranean climate conditions (Gjeloshi, 2007; Perovic *et al*., 1987). Despite the fact that kiwifruit find appropriate conditions in Albania, especially in plain and hilly areas of the Western Plain and Central part of Albania, after 20 years, cultivation area of kiwifruit remains moderate. According to INSTAT data, in 2013 in Albania, there were around 25 ha under cultivation, along Velipojë-Vlora coastal line, but this area is very little knowing the fact that climatic and terrain potential that our country possesses (Gjeloshi, 2007; Çeko *et al.*, 2002), eventhough, there is a trent for the increase of the area under cultivation. Since thea area under cultivation is increasing year-by-year, there is a need for high quality seedling production and new kiwifruit orchard construction. In Albania, as well as in othe countries, a big issue remains the seedling production with high quality. Seedling production of kiwifruit can be using traditional and moderm methods, such as seeds, vegetative cuttings (greewood, semi-hardwood and woody cuttings) and micropropagation. Any living vegetative plant tissue, cambium, epidermis, parenchyma of the bark, etc., can form roots if there are fulfilled appropriate environmental conditions and if there is a certain level of hormonal contents (growth regulators) (Hartman & Kester, 1990). The high hormone concentrations provoke the cambium and pericycle cell division from where the process of root formation starts. Roots originated from vegetative plant's parts under the hormone effect are similar to roots formed naturally (Hartman & Kester, 1990). Growth regulators (rooting phytohormones), such as Indole-3-Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) are being used for rooting acceleration of the vegetative cuttings (Rama, 2010). A higher concentration of the rooting hormones is used for woody and lignified parts, as well as, for those that hardly root, while very high concentrations of the rooting hormone inhibit the rooting process (Rama, 2010). Susaj *et al*., (2011) and Lekaj *et al*. (2011) have shown that the use different NAA concentration affected significantly the rooting percentage of the vegetative cuttings of thuya "Emeraud", as well as the seedling quantity.

Some early workers (Bhattacharya *et al*., 1978) have reported that indole-3-butyric acid (IBA) and gibberelic acid (GA_3) enhanced the formation of roots on the stem cuttings of *Abelmoschus esculentus* Moench. Serious efforts are necessary for the set-up of basic theoretical and practical knowledge to meet the need for high quality seedling production. Kiwifruit seedling production using different phytohormones concentration and different mature cuttings under greenhouse controlled atmosphere conditions is going to be a promised method in Albania.

MATERIALS AND METHODS

Experimental design. The study was conducted during two consecutive years, 2011-2013, in a greenhouse under the ownership of Gjovalin Gjeloshi in Lezha, equipped with an automatic fog system, on a perlite-peat (60:40) mix supporter (Kozai, 1991), with basal heating in March and without heating in the other planting periods. The air temperature in greenhouse was kept of 22-26^{o}C, basal perlite-peat mixture temperature of 17-18^{o}C, and air humidity of 90-95%. A randomized complete block design (RCBD) with five variants and three replications for variant, with a plot size of 100 cuttings for variant on each replication, was used. Variants were as below:

- V1 = hardwood (fully matured) cuttings, cutted in 5th January and stored in sand under blackness conditions for 60 days, placed for rooting in 5th March;
- V2 = hardwood cuttings, cutted in 5th February and stored in sand under blackness conditions for 30 days, placed for rooting in 5th March;
- V3 = hardwood cuttings, cutted and placed immediately for rooting in 5th March;
- V4 = semi-hardwood (half-matured) cuttings, cutted and placed immediately for rooting in 5th September;
- V5 = semi-hardwood cuttings, cutted and placed immediately for rooting in 5th October.

Hardwood cuttings and semi-hardwood cuttings of each variant were treated with 8 different indole-3-butyric-acid crystalline solution concentrations (0-5000 ppm) as is desciribed below.

Plant material. For planting, there were used hardwood and semi-hardwood cuttings with a length of 10-12 cm (3-4 nodes), taken from the middle part of the one year shoots (hardwood cuttings) and annual growth (semi-hardwood cuttings), respectively. Cuttings were taken from healthy mother plants of 8-10 years old, cultivar "Hayward". As was mentioned above, cuttings placed for rooting on 5th March were cutted and stored in sand under under blackness conditions for different periods (0, 30 and 60 days), and semi-hardwood cuttings were cutted and placed for rooting immediately on the respective day (5th September and 5th October), each year of study. Hardwood cuttings were without leaves, since they came from winter pruning, while semi-hardwood cuttings were left with 2 leaves (1-2 others were removed).

Just before the cuttings were placed for rooting on the rooting supporter, they were treated with eight different rates of indole-3-butyric-acid (IBA) crystalline concentration solutions (0 ppm, 1000 ppm, 2000 ppm, 2500 ppm, 3000 ppm, 4000 ppm, and 5000 ppm), and IBA talc 2000 ppm. Pre-prepared IBA solution and IBA talc was poured into a Petri dish, and the bottoms of the vegetative cuttings were dipped for few seconds. After the treatment with IBA, the kiwifruit cuttings were placed for rooting on a perlite supporter with basal heating. There were used 100 vegetative cuttings for each variant in each replication, planted in distances 10 cm x 5 cm, planting 200 cuttings per m^2. Planting of the vegetative cuttings for rooting was carried out at the same period two consecutive years.

Measurements and observations. There were observed, measured and evaluated several future seedling features. Twenty days after planting, there were observed the formation of root nodules and callus ring at the end of cuttings, and fortyfive days later, consisting of 65 days after planting, there were counted all rooted cuttings and was evaluated the rooting percentage for each variant and replication. In this time, cuttings were transferred in 200 cc vases and were placed under natural environmental conditions for acclimatization. Root length (cm) was measured 65 days after planting,

and vine length (cm) was measured 80 days after planting for all rooted cuttings for all variants. Measurements for root length (cm) and vine length (cm) were carried out in all rooted cuttings. Measured data were recorded, analyzed and were calculated mean values, which served for comparison between variants and treatments.

Statistical analyses. The obtained data were subject of ANOVA and differences between variants were tested using LSD test ($\alpha = 0.05$) (Papakroni, 2001).

RESULTS AND DISCUSSIONS

Rooted cuttings and rooting percentage of hardwood and semi-hardwood cuttings treated with different IBA concentration

There were counted rooted cuttings and was evaluated the rooting percentage for each variant in each replication, sixtyfive days after planting for rooting. In this time, cuttings were transferred in 200 cc vases and were placed under natural environmental conditions for acclimatization. Observed results showed significant differences between variants (hardwood and semi-hardwood cuttings and planting periods) and IBA treatments, as well. Results showed that hardwood cuttings variants, placed for rooting in 5th March, rooted better than semi-hardwood cuttings placed for rooting in 5th September or 5th October, despite the storage period. Use of Indole-3-Butyric Acid at any concentration significantly affected rooting percentage of the kiwifruit cuttings, placed for rooting in three different planting periods, but the highest results were achieved using IBA 3000 ppm for all variants (Table 1). The best results were achieved for variant 3 using hardwood cuttings, cutted in 5th March and placed immediately for rooting in 5th March, treated with IBA crystalline (3000 ppm solution) by 92%, followed by variant 2 (hardwood cuttings cutted in 5th February and stored in sand under blackness conditions for 30 days, placed for rooting in 5th March) treated with IBA crystalline (3000 ppm solution) by 82%, and variant 1 (hardwood cuttings cutted in 5th January and stored in sand under blackness conditions for 60 days, placed for rooting in 5th March) treated with IBA crystalline (3000 ppm solution) by 76%. The increase of rooting percentage for V3 (hardwood cuttings cutted and placed immediately for rooting in 5th March), treated with IBA crystalline solution 3000 ppm, was 84% higher compare to V3 with untreated cuttings. Results of all variants using IBA crystalline solution 2500 ppm and IBA talc 2000 ppm followed the same patern as IBA crystalline solution 3000 ppm, but with significantly lower results (Table 1).

Table 1. Rooted cuttings and rooting percentage of different vegetative cuttings, planted in different periods of the year and treated with different IBA concentration (mean values, different superscript letters indicate significant difference between variants and IBA concentration at $p<0.05$).

Variants	PC total	RC RP (%)	IBA crystalline solution concentration							IBA talc
			0 ppm	1000 ppm	2000 ppm	2500 ppm	3000 ppm	4000 ppm	5000 ppm	2000 ppm
V1	300	RC	12 ^{fB}	30 ^{eC}	54 ^{dBC}	114 ^{cC}	228 ^{aC}	48 ^{dC}	18 ^{eB}	120 ^{cCD}
		RP (%)	4%	10%	18%	38%	76%	16%	6%	40%
V2	300	RC	12 ^{gB}	48 ^{fB}	66 ^{eB}	144 ^{bB}	246 ^{aB}	72 ^{dB}	24 ^{gAB}	132 ^{bB}
		RP	4%	16%	22%	48%	82%	24%	8%	

		(%)								44%
V3	300	RC RP (%)	24 fA 8%	66 eA 22%	90 dA 30%	168 cA 56%	276 aA 92%	96 dA 32%	36 fA 12%	192 bA 64%
V4	300	RC RP (%)	0 0%	24 eD 8%	66 dB 22%	96 cD 32%	204 aD 68%	36 eC 12%	12 efC 4%	132 bB 44%
V5	300	RC RP (%)	0 0%	18 fD 6%	48 dC 16%	78 cE 26%	120 aE 40%	30 eD 10%	12 fC 4%	108 bD 36%
LSD (0.05) = 13.9										

Note: PC = Planted cuttings; RC = Rooted cuttings; RP = Rooting percentage

Higher results of rooting percentage for variant 3 showed that there is a need for a specific amount of winter low temperatures under 5-7^{o}C for digestion of blocking inhibitors of liquids moving in kiwifruit cuttings (Bellini and Monastra, 1986).
Observed results were similar to Gjeloshi *et al*. (2013), Çeko *et al.,* (2004), Biasi *et al*. (1990), and Revilla *et al.* (1991) with kiwifruit cuttings and Susaj *et al*., (2011) and Lekaj *et al*. (2011) with vegetative cuttings of thuya "Emeraud". Variant 3, using hardwood cuttings, cutted and placed immediately for rooting in 5th March, treated with IBA crystalline solution 3000 ppm, must be recommended for kiwifruit seedling producers in order to achieve the best rooting results (Gjeloshi *et al.*, 2014; Ferraj *et al.,* 2014; Gjeloshi *et al*. (2013).
Root length (rooting system development) ***(cm)***. Measurements of root length (cm) of rooted cuttings were carried out 65 days after planting, at the time when rooted cuttings were transferred in 200 cc vases and were placed under natural environmental conditions for acclimatization. Measurements for root length (cm) and vine length (cm) were carried out in all rooted cuttings. Measured data were recorded, analyzed and were calculated mean values, which served for comparison between variants and treatments. There was observed that different used cuttings and different IBA crystalline concentration solutions significantly affected the root development (root length – RL, cm) of rooted cuttings (seedlings), in three planting periods. There were observed significant differences between hardwood cuttings and semi-hardwood cuttings for both untreated and treated variants.
Observed results showed that seedlings comming from hardwood cuttings variants, placed for rooting in 5th March (despite the storage period), were with a better quality than seedlings comming from semi-hardwood cuttings placed for rooting in 5th September and/or 5th October. The highest quality seedlings were achieved for variant 3, using hardwood cuttings, cutted and placed immediately for rooting in 5th March, treated with IBA crystalline solution 3000 ppm, where for 76.8% of the seedlings was measured a root length over 15 cm, followed by variant 3 (hardwood cuttings cutted and placed immediately for rooting in 5th March, treated with IBA crystalline solution 3000 ppm) by 71.4%, variant 2 (hardwood cuttings cutted in 5th February and stored in sand under blackness conditions for 30 days, placed for rooting in 5th March) by 66.7%, and variant 1 (hardwood cuttings cutted in 5th January and stored in sand under blackness conditions for 60 days, placed for rooting in 5th March) by 57.9%, respectively, with a root length over 15 cm (Table 2). High quality seedlings were achieved also using hardwood cuttings placed for rooting in 5th March treated with

IBA talc 2000 ppm (despite the storage period), by 62.5%, 56.1% and 48.3% of seedlings with a root length over 15 cm (Table 2).

Vine length (cm). Measurements of vine length of rooted cuttings was carried out 80 days after planting or 15 days after rooted cuttings were transfered under natural environmental conditions for acclimatization. Vine length (cm) was measured for all rooted cuttings in all variants and replications. Measured data were recorded, analyzed and were calculated mean values, which served for comparison between variants and treatments. There was observed that different used cuttings and different IBA crystalline concentration solutions significantly affected the vine development (vine length – VL, cm) of rooted cuttings (seedlings), in three planting periods. There were observed significant differences between hardwood cuttings and semi-hardwood cuttings for both untreated and treated variants. Observed results showed that seedlings comming from hardwood cuttings variants, placed for rooting in 5th March (despite the storage period), were with a better quality than seedlings comming from semi-hardwood cuttings placed for rooting in 5th September and/or 5th October. The highest value for vine length was measured for variant 3, using hardwood cuttings, treated with IBA crystalline solution 3000 ppm, where 71.1% of the seedlings showed a vine length over 20 cm, followed by variant 3, using hardwood cuttings, treated with IBA talc 2000 ppm, where 62.5% of the seedlings showed a vine length over 20 cm; variant 2 (hardwood cuttings cutted in 5th February and stored in sand under blackness conditions for 30 days, placed for rooting in 5th March) where 59.4% of the seedlings showed a vine length over 20 cm; and so on (Table 3).

An interesting fact was that variants 4 and 5, treated and untreated with IBA solution or talc, showed lower rooting percentage, lower root length, and lower vine length compare to hardwood cuttings variants. Higher quality seedlings, with normal rooting system and vine development, were achieved using hardwood cuttings cutted and placed immediately for rooting in 5th March treated with IBA crystalline solution 3000 ppm.

After a period of 5-6 months of acclimatization under natural conditions, seedlings were ready to be planted in the field, in their permanent place.

a) b)

Figure 1. a) Hardwood cuttings cutted and placed immediately for rooting in 5th March; b) semi-hardwood cuttings cutted and placed immediately for rooting in 5th October.

Table 2. Root development (root length – cm) of hardwood and semi-hardwood cuttings, planted in different periods of the year and treated with different IBA concentration (two years mean values).

Variants	IBA solution concentration																					IBA talc		
	0 ppm			1000 ppm			2000 ppm			2500 ppm			3000 ppm			4000 ppm			5000 ppm			2000 ppm		
	RC	≤15 cm	>15 cm	RC	≤15 cm	>15 cm	RC	≤15 cm	>15 cm	RC	≤15 cm	>15 cm	RC	≤15 cm	>15 cm	RC	≤15 cm	>15 cm	RC	≤15 cm	>15 cm	RC	≤15 cm	>15 cm
V1	12	12	0	30	16	14 46.7%	54	34	20 37.4%	114	74	40 35.1%	228	106	122 57.9%	48	36	12 25%	18	16	2 11.1%	120	62	58 48.3%
V2	12	12	0	48	30	18 37.5%	66	42	14 36.4%	144	64	80 55.6%	246	82	156 66.7%	72	48	24 33.3%	24	12	12 50%	132	58	64 56.1%
V3	24	16	8 33.3%	66	30	36 45.5%	90	42	48 53.3%	168	48	120 71.4%	276	64	212 76.8%	96	60	36 37.5%	36	12	24 66.7%	192	72	120 62.5%
V4	0	0	0	24	18	6 25%	66	54	12 18.2%	96	78	18 18.8%	204	174	30 14.7%	36	30	6 16.7%	12	12	0	132	120	12 9.1%
V5	0	0	0	18	18	0	48	48	0	78	60	18 23.8%	120	96	24 20%	30	30	0	12	12	0	108	96	12 11.1%
LSD (0.05) = 4.85																								

Table 3. Vine length (cm) of hardwood and semi-hardwood cuttings, planted in different periods of the year and treated with different IBA concentration (two years mean values).

Variants	IBA solution concentration																					IBA talc		
	0 ppm			1000 ppm			2000 ppm			2500 ppm			3000 ppm			4000 ppm			5000 ppm			2000 ppm		
	RC	≤20 cm	>20 cm	RC	≤20 cm	>20 cm	RC	≤20 cm	>20 cm	RC	≤20 cm	>20 cm	RC	≤20 cm	>20 cm	RC	≤20 cm	>20 cm	RC	≤20 cm	>20 cm	RC	≤20 cm	>20 cm
V1	12	12	0	30	24	6 20%	54	44	10 18.5%	114	74	40 35.1%	228	130	88 42.9%	48	28	20 41.7%	18	14	4 22.2%	120	68	52 43.3%
V2	12	12	0	48	30	18 37.5%	66	40	26 39.4%	144	84	60 41.7%	246	100	146 59.4%	72	36	36 50%	24	16	8 33.3%	132	64	68 51.5%
V3	24	18	6 25%	66	30	36 54.6%	90	48	42 46.7%	168	78	90 53.6%	276	80	196 71.1%	96	46	50 52.1%	36	20	16 44.4%	192	72	120 62.5%

V4	0	0	0	24	18	6 25%	66	54	18.2%	96	78	18 18.8%	204	174	30 14.7%	36	30	6 16.7%	12	12	0	132	80	52 39.4%
V5	0	0	0	18	18	0	48	48	0	78	60	18 23.1%	120	96	24 20%	30	30	0 0	12	12	0	108	96	12 11.1%
LSD (0.05) = 5.67																								

CONCLUSIONS

Hardwood and semi-hardwood cuttings, planted in different periods of the year, singnificantly affected the rooting percentage, root system development and vine growth of kiwifruit seedlings. Hardwood cuttings, placed for rooting in 5th March showed the highest rooting percentage, the highest root system development and the highest vine length, despite the cutting storage period. The highest rooting percentage was observed for variant 3 using hardwood cuttings cutted and placed immediately for rooting in 5th March, treated with IBA crystalline solution 3000 ppm, by 92%. Variant 3 showed to have the highest quality seedlings, where, sixtyfive days after planting, 76.8% of the seedlings (rooted cuttings) showed a root length over 15 cm, and, 80 days after planting, 71.1% of the seedlings showed a vine length over 20 cm.
In order to achieve the best quality seedlings of kiwifruit, with a strong rooting system and normal vine growth, we recommend using hardwood cuttings cutted and planted immediately in 5th March, treated with IBA crystalline solution 3000 ppm.

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