Rooting of blackberry (*Rubus fruticosus* L.) hardwood cuttings as influenced by cutting time and cutting length

Iman Jaza Baqi Hawrami and Ibrahim Maaroof Noori

College of Agricultural Engineering Sciences/ University of Sulaimani/ Kurdistan Region/ Republic of Iraq.

Corresponding author: eman.baqi@univsul.edu.iq

DOI: https://doi.org/10.36077/kjas/2024/v16i1.10727

Received date: 12/12/2022

Accepted date: 16/1/2023

Abstract

This study was carried out during 2021-2022, in the Department of Horticulture, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaymaniyah, Kurdistan Region-Iraq, to find out the effects of cutting time (T1: Mid-Nov., T2: Mid-Dec., T3: Mid-Jan., and T4: Mid-Feb.), cutting length (L1: 10 cm and L2: 20 cm), and their interactions on rooting and some growth parameters of blackberry *Rubus fruticosus* L. hardwood cuttings. The local blackberry cuttings were transplanted in a low tunnel greenhouse at Zarrayen, Sulaymaniyah governorate. Plastic pots of 28 cm radius x 26 cm height, containing a substrate (peat moss: perlite: builder's sand with a 1:1:1 v/v ratio) were used, and 6 cuttings were planted as a replicate out of three replicates. The experimental design was laid out in a factorial complete randomized design (CRD), the data were subjected to the Analysis of Variance (ANOVA), and the means were separated by Duncan's multiple range test ($P \leq 0.05$). After 90 days, the results showed that T1 was significantly superior in rooting (95.83%), shoot dry weight (2.03 g), shoot length (24.68 cm), and average leaf area (2.22 cm^2). L2 cuttings recorded the highest significant values of rooting (96.87%), shoot dry weight (1.83 g), root dry weight (0.56 g), shoot length (25.49 cm), number of lateral branches per cutting (18.52), and average leaf area (2.19 cm^2). The interaction (T1+L2) was significantly superior in rooting (98.61%), shoot dry weight (3.02 g), shoot length (28.41 cm), root length (51.04 cm), and average leaf area (2.47 cm^2)

Keywords: *Rubus fruticosus* L., Hardwood cuttings, Rooting, Leaf area, Chlorophyll content.

Introduction

Blackberries often called "Brambles" are a diverse group of species and hybrids in the genus *Rubus*, which belongs to the rose family (*Rosaceae*). *Rubus* is considered one of the most diverse genera of flowering plants and is adapted to a wide range of environmental conditions, they are native to Asia, Europe, North and South America, however, blackberries grown in specific regions are derived from species indigenous to that region (1) comprising more than 700 species (6). *Rubus fruticosus* L., which also grows wild in Iraqi Kurdistan, is a shrub famous for its fruit, which is traded globally due to its delicious taste, pleasant flavour and nutritional profile. These fruits are consumed fresh or processed to make food products such as jam, jelly, wine, tea, ice cream, desserts and bakery products (39). The cooked roots are also used as food (21), while fresh or dried leaves are used as a tea (27), and the young shoots are eaten as salads after peeling (22). Blackberry fruit contains high levels of anthocyanins and other phenolic compounds, mainly flavonols and ellagitannins, which contribute to its high antioxidant capacity and other biological activities. Blackberry phenolic compounds have protective effects on age-related neurodegenerative diseases and bone loss and can inhibit low-density lipoprotein and liposomal oxidation. Blackberry extracts have also exerted antimutagenic effects by modifying cell signalling pathways and suppressing tumour promotion factors (19).

Blackberries are propagated by many vegetative propagation methods, i. e. suckers, tip layering, leaf-bud cuttings, root cuttings, semi-hardwood and hardwood cuttings in addition to tissue culture (14). Suckers need a huge amount of plant material, trailing type blackberries which do not sucker can be propagated by tip layering which needs a lengthy production period plus large spaces and plant material with few propagules, leaf-bud cuttings need bottom heat and intermittent mist, root cuttings need excessive effort, hand labor and indulge the stock plants, tissue culture also needs high techniques, expensive equipment and faces many problems (9). Hence, among all vegetative methods, hardwood cuttings besides some difficulties in rooting, but plant material that could be obtained after pruning with the capability to produce more propagules, may remain most suitable (1 and7).

Cutting time in the majority of horticultural plants is considered important for better rooting since the physiological status relating to growth, performance, and rooting capacity are all varied with varying seasons (14). Many studies were undertaken on different *Rubus* species and emphasized the importance of cutting season (11, 18, and 34). On the other hand, cutting length, which determines the number of lateral buds per cutting and also the amount of storage carbohydrates and other growth promoters, influences the rooting capacity and hence the growing degree of the transplant (14). The longer the cuttings the better the rooting capacity, while the less number of cuttings can be obtained from the stock plants. Any trying for rooting enhancement in shorter cuttings, and hence decreasing the necessity of stock plant material, is substantially considerable. In this aspect, very scarce scientific works with blackberries have been done. (37) investigated the effect of cutting length (5, 10, 15, and 20 cm) in black raspberries, and they found that cutting lengths between10-20cm obtained better results for rooting cuttings and subsequent growth parameters.

Although blackberries have wide acceptance in Kurdistan, it is not yet cultivated on a commercial scale. Hence, the objective of this study, as the first try toward making blackberry cultivation familiar, is to determine the best time for taking cuttings with two different cutting lengths and determining their role in the rooting capacity of the hardwood cuttings of wild-grown blackberries in Iraqi Kurdistan.

Material and Methods

This study was carried out during the period between November 2021 and July 2022, in the Department of Horticulture, College of Agricultural Engineering Sciences, University of Sulaimani/ Kurdistan Region-Iraq.

The cuttings of the local blackberry were taken from a natural groove located in Khurmal, Sulaymaniyah governorate with 35° 18' 18" N, 46° 02' 14" E, and 558 m AMSL. While the cuttings were transplanted in a low tunnel greenhouse located in Zarayan, Sulaymaniyah governorate with 35° 19' 06" N, 45° 40' 19’’, E and 544 m AMSL.

A planting media was prepared from (peat moss + perlite + builder’s sand) with a (1:1:1) v/v ratio, however the black plastic pots with 28 cm radius x 26 cm height, were used for planting the cuttings, in each pot, 6 cuttings were planted as one replicate out of three replicates per treatment combination. The cuttings were taken from a natural groove at Khurmal on four different dates (Mid-November, Mid-December, Mid-January, and Mid-February). On each date, two lots of 10 and 20cm hardwood cuttings, with 0.5-1.0 cm diameter, were taken from the bases of current seasons’ growths of blackberry primo cane shoots, using sharp secateurs, sterilized continuously with ethyl alcohol sprays. The cuttings were taken making a straight cut at 0.5 cm below the lowest node and a slant cut about 1 cm above the highest node then the prepared cuttings were wrapped with sterilized polyethylene sheets and transferred as quickly as possible to the greenhouse so as to be planted in the same day of taking them. The parameters that were considered, included rooting%, shoot dry weight (g), root dry weight (g), shoot length (cm), root length (cm), number of lateral branches per seedling, average leaf area (cm^2) (12) and total chlorophyll content of leaves ($mg.g^{-1}$ on a fresh weight basis) (38).

The experimental design which included two factors: Four times of taking cuttings and two cutting lengths, was laid down in a factorial complete randomized design (CRD), the data were subjected to the Analysis of Variance (ANOVA), and mean separations were done using Duncan’s multiple range test (P≤0.05) and the whole data processing was done using (XLSTAT) (2).

Results and Discussion

Rooting%

Regarding the different cutting times (Table 1), it was found that T1 got the superiority in rooting percentage of (95.83%) which was significantly higher than that of all other times of taking cuttings. Meanwhile, the lowest significant average of percent rooting (72.92%) was observed from T2. These results might be attributed to the differences in carbohydrate contents of the cutting tissues which act as the main sources of energy needed for root formation (4). Many researchers emphasize the difference in the rooting capacity of cuttings taken at different times (10, 16, 18), this could be related to the difference in the hormonal balance that favors the rooting of hardwood cuttings taken at different times (8).

Twenty-centimeter cuttings again caused a significantly higher value (96.87%) of rooting percentage of blackberry cuttings compared to ten-centimeter cuttings which gave only 63.89% rooted cuttings. There are no studies in the literature involving *Rubus* species evaluating the effect of different sizes and lengths of cutting on rooting (5). These results are in consistent with the results of other studies conducted in other genera, (34) noticed that two-node non-leafy cutting of blackberries with more stored carbohydrates performed better-rooting results than one-node cuttings. Additionally, (37) also concluded that the highest rooting percentage (58.89 %) was recorded in apple cuttings of length 35 cm compared to (15, 25, and 45 cm) lengths. Furthermore, (21) reported that herbaceous cuttings 5 cm in length of blueberry ‘Woodard’ showed a higher percentage of rooting (80%) compared to 12cm cuttings (20%).

Table 1. Effect of cutting time, cutting length and their interactions on rooting% of blackberry (*Rubus fruticosus* L.) hardwood cuttings

Cutting time	Cutting length (cm)		Means of cutting time
	10-cm	20-cm	
T1 (Nov. 15)	93.054 a †	98.611 a	95.833 a
T2 (Dec. 15)	48.612 b	97.222 a	72.917 b
T3 (Jan. 15)	58.334 b	94.443 a	76.389 b
T4 (Feb. 15)	55.555 b	97.222 a	76.388 b

Means of cutting length	63.889	b	96.874	a

† Means with the same letters are not significantly different according to Duncan's multiple ranges test (P≤0.05).

Dual effects of cutting times (T1, T2, T3, and T4) and cutting length (10 and 20 cm) showed that 20-cm cuttings taken at all times gave higher values of rooting percentage compared to 10-cm cuttings within all times except T1. The lowest significant rooting percentage (55.56%) of cuttings was noticed from 10cm cuttings taken in T4. This emphasizes that cutting length is a key factor to enhance rooting, the longer cuttings gave better results, regardless of the time of taking cuttings. According to (13) the real importance of carbohydrates for root formation is that auxin requires a source of carbon for the biosynthesis of nucleic acids and proteins, leading to the need for energy and carbon for root formation. (33) Observed that temperature has a direct effect on plant metabolism, which may have favored root development. According to (14), under suitable conditions (high humidity and heat), the rooting of cuttings is easy and reaches high percentages. This may be regarded as a considerable factor in cutting the propagation of blackberries. Cutting length alongside cutting time could be exploited for this purpose without using chemicals and/or growth regulators. This result is in conformity with those found in many previous studies (15, 16, 17, 18, and 21).

Shoot dry weight (g)

As shown in (Table 2), regarding the different cutting times, T1 (November 15) gave the highest average of shoot dry weight (2.037 g) which was significantly superior over all other times except T4 (February 15) which gave (1.325 g), whereas the lowest significant average (1.015 g) was given by T3 (January 15). The same trend was also true in the previously mentioned parameters (sprouting and rooting percentages), hence, the same reasoning may also be true for this parameter (shoot dry weight), since time 1 (November 15) could be favorable for taking blackberry cuttings causing the superiority of the majority of parameters. This result is in conformity with those of many researchers (3, 16).

Concerning cutting length, 20-cm cuttings gave a higher significant value (1.836 g) compared to 10-cm cuttings which gave (0.867 g) average shoot dry weight. Van der Krieken *et al*,(35) reported that different size of cuttings with respect to length and diameter has exhibited a significant influence on the rooting of cuttings.

The interaction effects between cutting time and cutting length, 20-cm cuttings taken on T1 got the superiority in average shoot dry weight with (3.024 g) compared to all other interactions. However, the lowest significant effect was observed in the interaction between T2 and 10-cm cuttings with a mean value of (0.452 g). This result is in agreement with the findings of Lust (21) who found a positive correlation between cutting time and length in blueberry cutting propagation. Additionally, according to Pacurar *et al*,(26), the formation of adventitious roots is a complex genetic trait regulated by interactions between environmental and endogenous factors.

Table 2. Effect of cutting time, cutting length and their interactions on shoot dry weight (g) of blackberry (*Rubus fruticosus* L.) hardwood cuttings

Cutting time	Cutting length (cm)				Means of cutting time	
	10-cm		20-cm			
T1 (Nov. 15)	1.049	b †	3.024	a	2.037	a
T2 (Dec. 15)	0.452	b	1.609	b	1.015	b
T3 (Jan. 15)	0.745	b	1.285	b	1.031	b
T4 (Feb. 15)	1.223	b	1.427	b	1.325	ab
Means of cutting length	1.867	b	1.836	a		

† Means with the same letters are not significantly different according to Duncan's multiple ranges test (P≤0.05).

Root dry weight (g)

As indicated in (Table 3), concerning the different cutting times, although cuttings taken on T4 (15 February) gave the higher average root dry weight (0.479 g), and the lower value (0.377 g) has resulted from T1 (15 November), these differences were not significant.

Regarding cutting length, 20-cm cuttings gave a higher significant value (0.569 g) of root dry weight compared to 10-cm cuttings which gave (0.313 g). This result is in agreement with the result found by (36) who recorded the maximum dry weight of roots in apple cuttings (2.09 g) in cuttings of length 35 cm, then cuttings of (14, 25, and 45 cm), whereas, this result is controversial with (20) who concluded that compared to 8 and 12 cm, cuttings of 'Woodward' and 'Brite Blue" cultivars of blueberry with 5 cm length performed better.

The interaction effects of cutting times (T1, T2, T3, and T4) and cutting length (10 and 20 cm) showed that 20-cm cuttings of T3 gave the higher significant value of average root dry weight (0.639) without significant difference from the other 20-cm cuttings either treated or non-treated with IBA. The lowest significant average of root dry weight (0.239 g) was noticed from 10cm cuttings of T2. This might be related to the effect of more carbohydrate reserves in the longer cuttings, and on the other hand to the proper environment for the growth of roots (14). The result is in harmony with the findings of Higuchi *et al*, (15) and Hussain *et al*, (17). However, this result is in contrast with what was revealed by Koyama (20) who stated that the shorter cuttings led to a higher root dry mass.

Table 3. Effect of cutting time, cutting length and their interactions on root dry weight (g) of blackberry (*Rubus fruticosus* L.) hardwood cuttings

Cutting time	Cutting length (cm)				Means of cutting time	
	10-cm		20-cm			
T1 (Nov. 15)	0.259	c †	0.494	ab	0.377	a
T2 (Dec. 15)	0.239	c	0.620	a	0.430	a
T3 (Jan. 15)	0.318	bc	0.639	a	0.578	a
T4 (Feb. 15)	0.434	abc	0.524	a	0.479	a
Means of cutting length	0.313	b	0569	a		

† Means with the same letters are not significantly different according to Duncan's multiple ranges test (P≤0.05).

Shoot length (cm)

Table 4 shows that cutting time caused significant differences in average shoot length mean values, since T1 (November 15) gave the highest value (24.688 cm) which was significantly higher than all other three times afterward except T3 with (21.514 cm), whereas T2 (December 15) with (18.688 cm) gave the lowest significant value. This may be caused by the same reasons mentioned previously for the other characteristics of the present study, i.e.; sprouting and rooting percentages, and shoot and root dry weights. On the other hand, the results are in agreement with those of previous studies such as Bueno *et al*,(8), Kahramanoğlu and Umar(17), Kang *et al*,(18), and Mehta *et al*,(24) who reported that the cuttings should be collected earlier so as to be succeeded.

Twenty-centimeter cuttings caused a significantly higher value (25.496 cm) of average shoot length of blackberry cuttings compared to ten-centimeter cuttings which gave only (17.610 cm) average shoot length. This can also emphasize that, to some extent, the longer the cuttings, the longer the shoots emerged (14). This result is consistent with many previous studies' results, i. e.; Balestrin *et al*, (5) showed that 20-cm cuttings of *Rubus erythroclados* gave 52.7 cm shoot length which was significantly different compared to 10 and 15cm cuttings giving 18.7 and 41.8 cm shoot lengths, respectively. Kahramanoğlu and Umar (17) also indicated that the length of shoots that emerged from pomegranate cuttings is proportional to cutting length.

Table 4. Effect of cutting time, cutting length and their interactions on shoot length (cm) of blackberry (*Rubus fruticosus* L.) hardwood cuttings

Cutting time	Cutting length (cm)		Means of cutting time
	10-cm	20-cm	
T1 (Nov. 15)	20.958 ab †	28.417 a	24.688 a
T2 (Dec. 15)	11.375 c	26.000 ab	18.688 b
T3 (Jan. 15)	18.208 bc	24.819 ab	21.514 ab
T4 (Feb. 15)	19.900 ab	22.750 ab	21.325 ab
Means of cutting length	17.610 b	25.496 b	

† Means with the same letters are not significantly different according to Duncan's multiple ranges test (P≤0.05).

The combinational effects between cutting time and cutting length, 20-cm cuttings taken in T1 got the superiority in average shoot length with (28.417 cm), but no significant difference occurred between this interaction and other interactions of 20cm cuttings taken on different dates. However, the lowest significant effect was observed in the interaction between T2 and 10-cm cuttings with a mean value of (11.375 cm). Other researchers found the same relationship between the time of cutting and cutting length in their effect on shoot length resulting in cutting propagation (17 and 18).

Root length (cm)

It is clear from (Table 5) that none of the three singular factors (cutting time and cutting) caused significant differences in this parameter, however, there were little significant differences due to the combinational effect, in which 20-cm cuttings taken on November 15th (T1) gave the higher significant value of root length (51.042 cm) compared to the different length cuttings taken on the other dates. Meanwhile, the lowest significant average root length (34.375 cm) of cuttings was noticed from 10cm cuttings taken on

December 15th (T2). Again, the same interaction, as mentioned in many previous parameters, caused the significant value of shoot length of blackberry cuttings, (28) reported that the highest temperature obtained at the time of collection in September, favored the greater length of roots. These results are in parallel with those found by Higuchi *et al*,(15), Hussain *et al*,(16) in blackberry; Kahramanoğlu and Umar(17) in pomegranate, and Koyama *et al*,(20) in blueberry cutting propagation .

Table 5. Effect of cutting time, cutting length and their interactions on root length (cm) of blackberry (*Rubus fruticosus* L.) hardwood cuttings

Cutting time	Cutting length (cm)				Means of cutting time	
	10-cm		20-cm			
T1 (Nov. 15)	39.708	bc †	51.042	a	45.375	a
T2 (Dec. 15)	34.375	c	42.792	abc	38.583	a
T3 (Jan. 15)	40.833	abc	45.667	ab	43.250	a
T4 (Feb. 15)	45.958	ab	39.417	bc	42.688	a
Means of cutting length	40.219	a	44.729	a		

† Means with the same letters are not significantly different according to Duncan's multiple ranges test (P≤0.05).

Number of lateral branches

The data shown in (Table 6) below, denotes the effect of cutting time, cutting length, and their interactions on the number of lateral branches per cutting in blackberry.

About cutting time, cuttings taken in T4 (February 15) gave a higher number of lateral branches (19.542) this average was higher significantly compared to cuttings taken in other times except for T3 with (15.708). The lowest mean value (5.542) was given by T1 (November 15). This might result from the favorable conditions of light, humidity, and temperature inside the greenhouse where the cuttings were planted which resulted in more shoot growths than root growth on one hand and on the other hand due to the imbalance between shoot and root systems since the last time of taking cuttings caused the formation of the least roots (31).

Twenty-centimeter cuttings again caused a significantly higher value (18.521) of the number of lateral branches per cutting compared to ten-centimeter cuttings which gave only 6.604 branches per cutting. This could be related to the mobilization of more sugars and nutrients at the base of cuttings, during adventitious root generation (5). This finding is the same as found by Balestrin *et al*,(5).

The interactions between cutting times (T1, T2, T3, and T4) and cutting length (10 and 20 cm) showed that both combinations (T4+20-cm and T3+20-cm) with the average values of (24.500 and 24.333), respectively got the significant superiority compared to the other combinations. However, the lowest significant number of lateral branches per cutting (1.750) was noticed from 10cm cuttings taken on T2 (15 December). For supporting these findings, no previous research works, neither on blackberry cuttings nor on other small fruits were obtained. Otherwise, our previously mentioned parameters may support or interpret these results, i. e.; since the first time of taking cuttings (T1, November 15) caused the longest shoots, it is normally awaiting that this time could result in the lowest number of lateral branches due to apical dominance (33).

Table 6. Effect of cutting time, cutting length and their interactions on the number of lateral branches per cutting of blackberry (*Rubus fruticosus* L.) hardwood cuttings

Cutting time	Cutting length (cm)		Means of cutting time
	10-cm	20-cm	
T1 (Nov. 15)	3.000 c †	8.083 c	5.542 b
T2 (Dec. 15)	1.750 c	17.167 b	9.458 b
T3 (Jan. 15)	7.083 c	24.333 a	15.708 a
T4 (Feb. 15)	14.583 b	24.500 a	19.542 a
Means of cutting length	6.604 b	18.521 a	

† Means with the same letters are not significantly different according to Duncan's multiple ranges test (P≤0.05).

Average leaf area (cm^2)

The data presented in (Table 7) indicate to the effects of cutting time, cutting length, and their interactions on the average single leaf area (cm^2) of blackberry cuttings.

Regarding the different cutting times, T1 (15 November) gave the highest average leaf area (2.220 cm^2) which was higher significantly compared to all other times, except T4 (15 February) which gave (2.093 cm^2). Alongside, the lowest significant average of leaf area (1.467 cm^2) was given by T2 (15 December). Many types of research indicate that cutting collection time which is positively correlated to environmental conditions of light, humidity, and average temperatures, and finally correlated to physiological status and hormonal balance between growth promoters such as auxins, gibberellins, and cytokine's and growth inhibitors such as abscisic acid and ethylene, has a great influence on the success of rooting the cuttings and hence subsequent growth characteristics, such as leaf area (3 and 10).

Twenty-centimeter cuttings gave a higher significant value (2.189 cm^2) of leaf area compared to ten-centimeter cuttings which gave (1.544 cm^2). For comparing our results regarding the leaf area of blackberry cutting seedlings, no previous studies were available.

Regarding the interaction effects between cutting time and cutting length, 20-cm cuttings taken on T1 got the significant superiority in the average of leaf area with (2.471 cm2), however, the lowest significant effect was observed in the interaction between T2 and 10-cm cuttings with the mean value of (1.073 cm^2). Since this interaction caused the highest significant values of the majority of studied parameters, i. e.; the process of adventitious root formation consequently terminated in the best results of plant growth in general and leaf area in particular, which may be influenced by a number of internal and external factors. Among the internal factors, the most important role is ascribed to phytohormones (32).

Table 7. Effect of cutting time, cutting length and their interactions on average leaf area (cm^2) of blackberry (*Rubus fruticosus* L.) hardwood cuttings

Cutting time	Cutting length (cm)				Means of cutting time	
	10-cm		20-cm			
T1 (Nov. 15)	1.968	ab †	2.471	a	2.220	a
T2 (Dec. 15)	1.073	d	1.860	bc	1.467	b
T3 (Jan. 15)	1.340	cd	2.033	ab	1.687	b
T4 (Feb. 15)	1.794	bc	2.392	ab	2.093	a
Means of cutting length	1.542	b	2.189	a		

† Means with the same letters are not significantly different according to Duncan's multiple ranges test (P≤0.05).

Leaf chlorophyll content (mg.g^{-1} on fresh weight basis)

Table (8) indicates that cutting time caused significant differences in leaf total chlorophyll content mean values, cuttings taken on T4 (February 15) gave the highest value (1.763 mg.g^{-1} fw), this value was significantly similar to that of T1 with (1.665 mg.g^{-1}), both of these were significantly superior onto the other two times of taking cuttings (T2 and T3). The lowest significant average of leaf chlorophyll content (1.259 mg.g^{-1}) was given by cuttings taken on T3 (January 15). As it was clarified in the previous table (9), there were no significant differences between T1 and T4, both of which gave the highest values of leaf area, the same tendency was also true for total leaf chlorophyll content, and it was reported that there is a positive correlation between leaf area and total chlorophyll of leaves (30). McDowell (23) also mentioned that the components of photosynthetic capacity are constrained by the amount and activity of the enzyme ribulose 1, 5-bis-phosphate electron transport capacity which is constrained by the number of thylakoid membranes. Environmental conditions also play a key role in plant growth performance before, during, and after taking the cuttings (25) which eventually affect the rooting and growth of the cuttings, and both times; T4 (February 15) and T1 (November 15) appear to be more suitable than the other two times (December 15 and January 15) which synchronized with winter months that seem to be unfavorable for taking blackberry cuttings, these results are conformity with those obtained by Bueno *et al*,(8), Hussain *et al*,16, Kahramanoğlu and Umar (17).

As for the cutting length, although twenty-centimeter cuttings with (1.593 mg.g^{-1}) total chlorophyll were maximal, it did not differ significantly from that of 10-cm cuttings with (1.445 mg.g^{-1}). Except for our previous data, in which the majority of parameters under the effect of cutting length emphasized the significance of longer (20cm) cuttings compared to shorter (10cm) cuttings, no previous studies were available regarding the chlorophyll content of leaves grown from cutting propagation of blackberries. Otherwise, there are many studies conducted on other plants that are in accordance with these results Bueno *et al*, (8), Hussain *et al*, (16) and Kahramanoğlu and Umar (17).

Table 8. Effect of cutting time, cutting length and their interactions on the leaf total chlorophyll contents (*mg.g*$^{-}$ f.w.) of blackberry (*Rubus fruticosus* L.) hardwood cuttings

	Cutting length (cm)	Means of

Cutting time	10-cm		20-cm		cutting time	
T1 (Nov. 15)	1.616	abc †	1.714	a	1.665	a
T2 (Dec. 15)	1.276	bc	1.503	abc	1.389	b
T3 (Jan. 15)	1.235	c	1.283	bc	1.259	b
T4 (Feb. 15)	1.655	ab	1.871	a	1.763	a
Means of cutting length	1.445	a	1.593	a		

† Means with the same letters are not significantly different according to Duncan's multiple ranges test (P≤0.05).

Concerning the interaction effects between cutting time and cutting length, it was found that the interaction (T4+20-cm) with 1.871 mg.g^{-1} was significantly superior, although the effect of this interaction was significantly similar to some other interactions. On the other hand, the lowest significant effect was observed in the interaction of (T3+10-cm cuttings) with the mean value of 1.235 mg.g^{-1}. This may be caused by the activation of more photosynthetic, which resulted in more chlorophyll contents (29) of blackberry leaves produced from longer cuttings (20-cm) and taken on the proper time (T1) which in turn caused the better interaction and hence resulted in better growth characteristics for the plants as whole.

Conclusion

From the results mentioned above, it was concluded that both time of cuttings and the length of cuttings had their roles in the enhancement of rooting capacity and hence the subsequent growth characteristics of hardwood cuttings of blackberry. November 15 was found to be more effective than any other time under this study in its effect either on the rooting percentage of the cuttings or subsequent growth and performance characteristics such as shoot and root length, their dry weights and vegetative growth which were represented as total leaf chlorophyll content, number of lateral branches and leaf area. Cutting length, however, had significant effects on the same characteristics, since it was revealed that 20-cm length led to better results in the majority of parameters than 10-cm cuttings.

Conflict of Interest

The authors have no conflict of interest.

References

1. **Abdrabboh, G.A.; S.M. Khalifa; H.F. Abdel Aziz and El-Rshdy, A. S.2021**. *In Vitro* Propagation of Blackberries (*Rubus sp*) Prim-Ark 45 Cultivar. 5th International Conference on Biotechnology Applications in Agriculture (ICBAA), Benha University, 8 April 2021, Egypt (Conference Online). Plant Biotechnology, 287-294.

2. **Addinsoftm.2016.**XLSTAT data analysis program for Windows software, https://www.xlstat.com
3. **Aref Hawramee, O.K.; R.R. Aziz and Hassan, D. A.**2019.Propagation of white mulberry *Morus alba* L. fruitless cultivar using different cutting times and IBA. IOP Conference Series: Earth and Environmental Science, 388(1): 1-11. DOI 10.1088/1755-1315/388/1/012069.
4. **Aslmoshtaghi, E. and A. R. Shahsavar.**2010.Endogenous soluble sugars, starch contents and phenolic compounds in easy and difficult-to-root olive cuttings. J. Biol Environ. Sci., 49 (11): 83–86.
5. **Balestrin, J.T.; T. Baroni and Rogalski, J.M.**2021. Effect of cutting size on the performance of exogenous auxin IBA in vegetative propagation of *Rubus erythroclados* Mart. ex Hook. f. Journal of Experimental Agriculture International, 43 (8): 56-66.
6. **Balslev, H. and J. L. Luteyn.1992.**Paramo, an Andean Ecosystem under Human Influence. Academic Press. London, UK.
7. **Bray, M.; C.C. Rom and Clark, J.R.2003**. Propagation of thornless Arkansas blackberries by hardwood cuttings. Discovery. The Student Journal of Dale Bumpers College of Agricultural. Food and Life Sciences. University of Arkansas System Division of Agriculture. 4: 9-13. USA.
8. **Bueno, P.M.C.; M.B.D. Tofanelli; W.A. Vendrame and Biasi, L.A.2021**. Paclobutrazol as an alternative to improve propagation of *Rubus brasiliensis* Mart. Scientia Horticulturae, 287, 110215. https://doi.org/10.1016/j.scienta.2021.110215
9. **Caldwell, J.D.1984**.Blackberry Propagation. HortScience, 19(2):193-195.
10. **Campagnolo, M.A. and R. Pio.2012**. Rooting of stem and root cuttings of blackberry cultivars collected in different times, cold storage and treatment with IBA. *Cienc. Rural.*, 42 (2): 232–237.
11. **Debner, A.R.; H. Hatterman-Valenti and Takeda, F.2019.**Blackberry propagation limitations when using Floricane cuttings. HortTechnology, 29 (3): 276-282. DOI: https://doi.org/10.21273/HORTTECH04266-18
12. **Drovnic, C.E.; G.S. Howell and Elore, A.J.1965**.Influence of crop load on photosynthesis and dry mater partitioning at several grapevines. II. Seasonal change in single leaf and whole vine photosynthesis. Amer. J. End. Vitic. 46 (40): 469-477.
13. **Fachinello, J.C.; A. Hoffmann and Nachtigal, J.C.2013.** Propagação de plantas frutíferas. 2nd ed. Brasília: Embrapa, 264 p.
14. **Hartmann, H.T.; D.E. Kester; F.T. Davies, Jr. and Geneve, R.L.2014.**Plant Propagation: Principles and Practices. 8th ed. Pearson Education Limited. USA.
15. **Higuchi, M.T.; L.T.M. Ribeiro; A.C. de Aguiar; D.M. Zeffa; S.R. Roberto and Koyama, R.2021**.Methods of application of indolebutyric acid and basal lesion on ‘woodard’ blueberry

cuttings in different seasons. Revista Brasileira de Fruticultura, 43(5):1-9.e-022 https://doi.org/10.1590/0100-29452021022.

16. **Hussain, I.; S.R. Roberto; R.C. Colombo; A.M. De Assis and Koyama,** R.2017.Cutting types colleceted at different seasons on blackberry multiplication. Revista Brasileira de Fruticultura, 39 (3): 1-8 (e939). http://dx.doi.org/10.1590/0100-29452017939.
17. **Kahramanoğlu, I. and Umar, K.2018**. Effects of cutting length, time and growing media on the sprouting of dormant semi-hardwood cuttings of pomegranate cv. Wonderful. African Journal of Agricultural Research, 13 (45): 2587-2590. DOI:10.5897/AJAR2018.13590
18. **Kang, Y.K.; M.R. Ko; S.Y. Kang and Riu, K.Z.2005.** Several factors affecting to rooting of stem cuttings in *Rubus buergeri* Miquel. Korean Journal of Medicinal Crop Science, 13 (3): 77-80.
19. **Kaume, L.; L.R. Howard and Devareddy, L.2012.**The Blackberry Fruit: A Review on Its Composition and Chemistry, Metabolism and Bioavailability, and Health Benefits. J. Agric. Food Chem., 60 (23): 5716–5727.
20. **Koyama, R.K.; A. de Assis; W. Borges; L. Yamamoto; R. Colombo; D. Zeffa; L. Barros; B. Barreira; I. Shahab; S. Ahmed and Roberto, S.2018**.Multiplication of blueberry mini-cuttings in different growth media. Agronomy Science and Biotechnology, 4(1):28-36.
DOI: https://doi.org/10.33158/ASB.2018v4i1p28
21. **Lust, J.1983.**The Herb Book; Bantam: New York. NY. USA.
22. **Malcolm, P.2012**.History of blackberry plants.
23. Available online: http://www.approvedarticles.com/Article/History-Of-Blackberry-Plants/1480 (accessed on 5 November 2012).
24. **McDowell, S.C**.2002.Photosynthetic characteristics of invasive and noninvasive species of *Rubus* (Rosaceae). American Journal of Botany, 89 (9): 1431-1438.
25. **Mehta, S.K.; K.K. Singh and Harsana, A.S.**2018.Effect of IBA concentration and time of planting on rooting in pomegranate (*Punica granatum*) cuttings. Journal of Medicinal Plants Studies, 6 (1): 250-253.
26. **Neocleous, D. and M. Vasilakakis**2007.Effect of NaCl stress on raspberry (*Rubus idaeus* L. 'Autumn Bliss'. Scientia Horticulturae, 112: 282-289.
27. **Pacurar, D.I.; I. Perrone and Bellini, C.**2014.Auxin is a central player in the hormone cross- talks that control adventitious rooting. Physiologia Plantarum, 151(1):83-96. doi: 10.1111/ppl.12171
28. Phillips, R.; Foy, N.1990.Herbs; Pan Books Limited: London. UK.
29. **Pizzatto, M., A. Wagner Júnior; D. Luckmann; K. Pirola; D.A. Cassol and Mazaro, S.M.** 2011.Influência do

uso de AIB, época de coleta e tamanho de estaca na propagação vegetativa de hibisco por estaquia. Revista Ceres, 58(4), 487–492.

30. **Rao, G.S.; I.A. Bisati; A. Sharma; S. Kosser and Bhat, S.A.2020**.Effect of IBA concentration and cultivars on number of leaves, leaf area and chlorophyll content of leaf in Pomegranate (*Punica granatum* L.) cuttings under temperate conditions of Kashmir. Journal of Pharmacognosy and Phytochemistry, 9 (SP 6): 86-90.
31. **Reich, P.B. and M. B. Walters.1994.**Photosynthesis-nitrogen relations in Amazonian tree species. II. Variation in nitrogen vis-a-vis specific leaf area influences mass-and area-based expressions. Oecologia, 97: 3-81.
32. **Schaff, S.D.; S.R. Pezeshki and Shields Jr, F.D.2002**.Effects of pre-planting soaking on growth and survival of black willow cuttings. Restoration Ecology, 10(2):267-274.
33. **Štefančič, M., F. Štampar and Osterc, G.2005.**Influence of IAA and IBA on root development and quality of *Prunus* 'Gisela 5' leafy cuttings. HortScience, 40: (7): 2052-2055.
34. **Taiz, L., E. Zeiger; I.M. Møller and Murphy, A.2015.Pl**ant physiology and Development, 6th ed. Sinauer Associates Incorporated. USA.
35. **Takeda, F., T. Tworkoski; C.E. Finn and Boyd, C.C.2011.**Blackberry propagation by non-leafy floricane cuttings. HortTechnology, 21(2):236-239.
36. **Van der Krieken, W.M.; H. Breteler; M.H. Visser and Mavridou, D.1993.**The role of the conversion of IBA into IAA on root regeneration in apple: introduction of a test system. Plant Cell Reports, 12 (4): 203-206.**Verma, P.; P.S. Chauhan; J.S. Chandel and Thakur, M.**2015.Effect of the size of cuttings (length and diameter) on rooting in cuttings of apple clonal rootstock Merton 793. Journal of Applied and Natural Science, 7(2):602-605. DOI:10.31018/jans.v7i2.652
37. **Villa, F.; D.R. Stumm; D.F. da Silva; F.J. Menegusso; G. Ritter and Kohler, T.R.2018**. Rooting of black raspberry with plant growth regulator. Ciencia Rural, 48(3):1-5 (e20161023).
38. **Wintermans, J.F.G.M. and De Mots, A.1965**.Spectrophotometric characteristics of chlorophyll a and b and their phenophytins in ethanol. Biochem. Biophys. Acta, 109: 448-453. (In: Knudson, L. L., Tibbitts, T. W. and Edwards, G. E. (1977). Measurement of ozone injury by determination of leaf chlorophyll concentration. Plant Physiol., 60: 606-608.).
39. **Zia-Ul-Haq, M.; M. Riaz; V. De Feo; H.Z.E. Jaafar and Moga, M.2014.**Rubus fruticosus L.: Constituents, Biological Activities and Health Related Uses. Molecules, 19: 10998-11029.
doi: 10.3390/molecules190810998