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Rooting of thornless blackberry cuttings as induced by the extract of white willow (*Salix alba* L.) shoots collected in different times

Kocher Omer Salih[1] **Aram Akram Mohammed**[1] **Ibrahim Maaroof Noori**[1]

[1]*Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaymaniyah, Kurdistan Region, IRAQ*

*Corresponding Author: aram.hamarashed@univsul.edu.iq

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ABSTRACT

The aqueous extract of *Salix* spp contains many compounds which may act as root-promoting agents in cuttings. S. alba is a deciduous tree containing variable phytochemicals, which vary throughout the year. So, in this study, one- and two-year-old shoots of S. alba were collected on the 15th of each month in the year 2022, extracted in 2% ethanol at 9 g.L-1, and placed in a water bath at 35 °C. Then, they were applied to thornless blackberry cuttings for 1.5 hr. The results explained that the highest rooting percentage (66.67%) was obtained in the cuttings soaked in the extract of willow shoots collected on the 15th of January. They were not significantly different from control cuttings, but they were different from the cuttings soaked in the extract of willow shoots collected on the 15th of August and October (33.33%). The majority of other shoot and root traits were high in the cuttings soaked in the extract of willow shoots collected on the 15th of December. The willow shoots collected on the 15th of January contained the lowest total phenols (51.4 µg.mL^{-1}) and total flavonoids (29.07 µg.mL-1). Moreover, the highest total phenols (57 µg.mL^{-1}) and IAA (365.17 µg.mL^{-1}) were recorded in the willow shoots collected on the 15th of March. However, each total flavonoids (44.96 µg.mL^{-1}) and salicylic acid (492.61 µg.mL^{-1}) were the highest in the willow shoots collected on the 15th of April. Generally, based on rooting percentage, it is advisable to collect willow shoots on the 15th of January and February for extraction and application to the thornless blackberry cuttings.

***Keywords*: ** Flavonoids, IAA, phenols, salicylic acid, rooting.

INTRODUCTION

Since the findings obtained by [1], it has been approved that auxin; indole acetic acid (IAA) is crucial in adventitious root formation in cuttings. They also found that exogenous synthetic IAA had the same effect when applied to the cuttings. However, auxin has not been admitted as a single agent responsible for rooting in the cuttings, because many other substances may interact with auxins to induce root formation, such as sugars, phenolic compounds, and oxidase enzymes [2]. Therefore, many attempts have been made to find natural sources of auxins and other substances that promote root formation in cuttings with lower costs and lesser harm to humans and the ecosystem [3]. Among the most popular natural extracts frequently used for cuttings to improve rooting are plant extracts, particularly white willow (*Salix alba* L.) extract.

White willow is a deciduous tree from the Salicaceae family growing up to 30 m. It is endemic to Europe, Asia, and North Africa [4, 5]. The aqueous extract of *Salix* spp contains many compounds that may act as root-promoting agents in cuttings, along with fungicidal, insecticidal, and antibacterial properties [6]. Earlier, [7] found root-promoting substances in the extract of softwood cuttings of *S. alba*, and he confirmed that these substances synergistically interact with IAA to improve rooting in the mug bean cuttings. Other researchers concluded that there were different results when applying the water extract of *Salix* spp to the cuttings of different species. The best results were achieved in lavender (softwood) and chrysanthemum (semi-hardwood) cuttings at 1.06 µl.L^{-1} willow bark extract [8]. Similarly, an extract of willow (*S. alba* L.) was successfully used as a natural rooting stimulator in mug bean cuttings [9]. However, 0.2% willow extract in gel form did not give a favorable rooting in stem cuttings of cannabis [10]. Besides, [11] observed that the extracts of *Salix babylonica* were not effective in increasing rooting in olive cuttings cv. 'Nabali'. Furthermore, *S. alba* is a deciduous tree, so phytochemicals and hormones in deciduous trees are variable throughout the year [12]. Tienaho *et al.* [13] reported that seasonal and environmental factors caused changes in phytochemical contents in the bark of *Salix spp*. Besides, [14] referred to that during the vegetative season, precisely from March to July, secondary metabolites declined in the bark of willow clones.

As for propagation of thornless blackberries, they are readily propagated via clonal methods, digging of canes, crown division, dormant hardwood cuttings, softwood cuttings, leaf-bud cuttings, suckers, root cuttings, and layering [15]. The selection of one method relies on the number of new plants needed and the type of blackberry. In this regard, digging of canes, crown division, suckers, and layering do not allow the generation of new plants in large numbers for commercial production, because a high number of mother plants are required [16]. Additionally, root suckers and root cuttings are not recommended for the propagation of chimeral thornless cultivars due to losing thornlessness [17].

Cuttings propagation is widely used to propagate blackberries; however, the reports have revealed that the rooting of hardwood or floricane cuttings of blackberries is limited and variable. Nonetheless, cultivar, the time of taking cuttings, using auxins, and the cuttings atmosphere have decisive roles in successfully rooting blackberry cuttings [18, 19]. In light of the above-mentioned reasons, in this study, willow shoots were collected at different times, extracted, and then applied to the cuttings of thornless blackberry to enhance rooting capacity.

Materials and Methods

The research was carried out in the College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan Region-Iraq to demonstrate the effect of willow shoot extract on the rooting of thornless blackberry cuttings as the shoots of willow were collected at different times (on the 15th of each month along the year).

Collection of willow shoots and extractions

The willow shoots (*Salix alba* L.) were collected from one- and two-year-old shoots on the 15th of each month in 2022, from a single tree. The shoots were cut into small pieces upon taking and dried in the shade, and then they were stored in plastic containers at room temperature until extraction.

Quantification of total phenols and flavonoids in willow shoots

The dried willow shoots collected at different times were ground in a blender and used to analyse total phenols and flavonoids according to [20].

Colorimetric quantification of IAA in willow shoots

Determination of endogenous IAA in willow shoots was conducted according to a slight modification in the methods described by [21, 22]. So, 1.5 g of dried and well-ground willow shoots were mixed well with 10 mL of Salkowski's reagent, shaken thoroughly, and left for 1 hr. After that, the mixture was filtered through a filter paper, and the absorbance was read at 530 nm. The concentration of IAA was found using the IAA standard curve and represented as $\mu g.mL^{-1}$.

Determination of salicylic acid in willow shoots

Salicylic acid was determined using a spectrophotometer as described by [23], with a slight modification. The samples were prepared by taking 0.05 g of dried and ground willow shoots, mixed with 1 mL ethanol at 20%, shaken for 20 minutes, then centrifuged for 13 minutes at 13000 rpm. The supernatants were collected, and 100 µL was taken and mixed with 0.1% of $FeCl_3$ in 1% HCl up to 3 mL. After 30 minutes, the absorbance was read at 540 nm, and salicylic acid concentration was found using the salicylic acid standard curve as $\mu g.mL^{-1}$.

Preparation of willow shoot extracts

The extraction was initiated by grinding the dried willow shoots in a blender. The ground willow shoots taken from each collecting time (9 g) were extracted separately in 1 L of 2% ethanol, placed in a water bath at 35 °C for 3 hrs., and refrigerated for 24 hrs. After that, the extracts were filtered through filter papers, and the filtered extracts were applied to the cuttings.

Taking and treatment of the cuttings

The cuttings were taken on February 12, 2023, from the basal part of one-year-old shoots with a length of 12 cm (containing at least three buds) and a diameter of 4-7.5 mm. After transferring to the laboratory, they were soaked in 54% topsin fungicide at 1.5 $mL.L^{-1}$ for 30 min. The cuttings, after the surface drying, were soaked in the extracts of the willow shoots, 300 $mg.L^{-1}$ salicylic acid (SA) in 2% ethanol, 300 $mg.L^{-1}$ indole acetic acid (IAA) in 2% ethanol, and the control cuttings just in 2% ethanol for 1.5 hr. The treated cuttings were stuck in sand media prepared in black plastic pots as seven cuttings per pot put inside a high tunnel. The experiment was laid out in randomized complete block design (RCBD) with three replications. The average minimum and maximum temperatures inside the high tunnel are shown in Figure 1.

Statistical analysis

The data were collected after 80 days (on May 3, 2023) from planting the cuttings. The measurements were rooting percentage, callusing percentage, death percentage, number of main roots, length of the longest root, shoot length, shoot diameter, leaf number, leaf area, chlorophylls *a* and *b* in the leaves, and survival percentage after transplanting. To calculate the survival percentage, the rooted cuttings were transplanted to a mixed media of fine sand + compost in polyethylene bags with a size of 8×20 cm, then they were placed in a lath-house for 45 days. The average minimum and maximum temperatures inside the lath-house were between (15.7- 33.4 ℃). Chlorophylls *a* and *b* were quantified according to [24]. The collected data were analyzed using XLSTAT software version 2019.2.2, one-way ANOVA-RCBD, and Duncan's multiple range tests ($P \leq 0.05$)

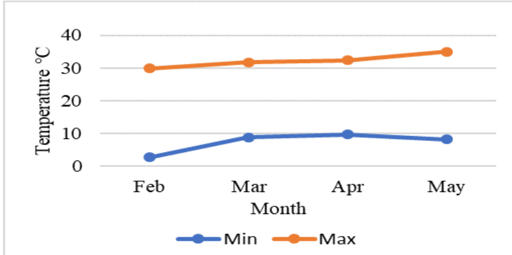

Figure 1. Average minimum and maximum temperatures inside the high tunnel, from February 12 till May 3, 2023

Results

Analysis of the data showed that the extract of willow shoots collected on different dates gave different rooting%, callusing%, death%, and root length (Table 1), but collection dates of willow shoots showed no significant differences in rooting%, callusing%, death%, and root length related to control cuttings. The highest rooting percentage (66.67%) was detected in the cuttings treated with the extract of willow shoots collected on the 15th of January, followed by the 15th of November (57.14%), and the 15th of February (52.38%). In contrast, the lowest rooting percentage (33.33%) was observed in the cuttings treated with the extract of willow shoots collected on the 15th of August and October and (38.09%) on the 15th of April and June. Control cuttings in this study gave 42.85% rooting. The callusing percentage was the highest (57.14%) in the treated cuttings with the extract of willow shoots collected on the 15th of December. However, it was the least (14.28%) in the treated cuttings with the extract of willow shoots collected on the 15th of January. No dead cuttings were observed in the ones treated with the extract of willow shoots collected on the 15th of December. Also, the willow shoot extraction taken from the collection date of the 15th of November showed the lowest death (4.76%) of the thornless blackberry cuttings. While the extract of the willow shoots collected on the 15th of

August resulted in the maximum number of dead cuttings (38.09%), followed by 15th of March (23.81%), and the treatment of 300 mg.L^{-1} SA (23.80%). Additionally, the longest root (25.89 cm) was achieved from the cuttings supplied with the extract of willow shoots collected on 15th of October. At the same time, the extract of the willow shoots collected on 15th of September and February caused the shortest roots (13.46 and 13.79 cm, respectively).

The data shown in the same table confirm that soaking thornless blackberry cuttings in extracts of willow shoots collected on

Table 1. Effect of 300 mg.L^{-1} of salicylic acid (SA), 300 mg.L^{-1} indole-3-acetic acid (IAA), and willow shoot extracts collected on different dates on rooting%, callusing%, death%, root number, root length, shoot length, and shoot diameter of thornless blackberry cuttings.

Treatments	Rooting%	Callusing%	Death%	Root length (cm)	Root number	Shoot length (cm)	Shoot diameter (mm)
Control	42.85 ab*	42.85 ab	14.28 ab	17.55 ab	6.93 cd	2.59 cd	1.98 a
SA 300 mg.L^{-1}	42.85 ab	33.33 ab	23.80 ab	17.63 ab	16.17 a	4.5 a-d	1.91 a
IAA 300 mg.L^{-1}	47.62 ab	38.09 ab	14.28 ab	15.19 ab	14.67 a	2.61 cd	1.85 a
15th Jan.	66.67 a	14.28 b	19.04 ab	18.07 ab	7.5 bcd	3.43 bcd	1.74 a
15th Feb.	52.38 ab	38.09 ab	9.52 ab	13.79 b	7.44 bcd	3.92 a-d	1.93 a
15th Mar.	42.85 ab	33.33 ab	23.81 ab	15.11 ab	4.33 d	2.52 cd	1.66 a
15th Apr.	38.09 b	42.85 ab	19.04 ab	20.96 ab	6.9 cd	3.88 a-d	1.7 a
15th May	47.61 ab	42.85 ab	9.52 ab	15.31 ab	8.53 bc	5.65 ab	1.87 a
15th Jun.	38.09 b	42.85 ab	19.05 ab	17.03 ab	7.62 bcd	4.97 abc	1.88 a
15th Jul.	47.61 ab	33.3 ab	18.95 ab	15.34 ab	4.67 d	4.79 a-d	1.77 a
15th Aug.	33.33 b	28.57 ab	38.09 a	17.51 ab	7.75 bcd	3.72 a-d	1.94 a
15th Sep.	47.62 ab	42.85 ab	9.52 ab	13.46 b	11.08 b	2.16 d	1.79 a
15th Oct.	33.33 b	47.61 ab	19.04 ab	25.89 a	6.37 cd	5.9 ab	1.95 a
15th Nov.	57.14 ab	38.09 ab	4.76 b	17.17 ab	9.87 bc	4.35 a-d	2.07 a
15th Dec.	42.85 ab	57.14 a	0 b	23.78 ab	14.8 a	6.23 a	2.02 a

* Values in the same column taken the same letter were not significantly different subjected to Duncan's multiple-range test at ($p \leq 0.05$).

different dates made significant differences in comparison with control cuttings regarding root number and shoot length. Soaking the cuttings in 300 mg.L^{-1} SA, the extract of willow shoots collected on 15th of December, and 300 mg.L^{-1} IAA increased root number to the highest values (16.17, 14.8, and 14.67, respectively). Whereas, the extracts of willow shoots collected on 15th of March and July gave the minimum number of roots (4.33 and 4.67, respectively). Similarly, the collection of willow shoots on 15th of December and their extract led to the longest shoot (6.23 cm). Shoot length was the shortest (2.16, 2.52, and 2.59 cm) in the cuttings supplied with the extract of willow shoots collected on the 15th of September and March, and in control cuttings, respectively. Furthermore, shoot diameter was not significantly different in all treated and control cuttings in this study.

The evaluation of the variance demonstrated that the extracts of willow shoots, which were collected on different dates, had a significant role in improving leaf number, leaf area, and the ratio of chlorophyll *a* and *b* when compared with control cuttings (Table 2). Leaf number was plentiful in the cuttings soaked in 300 $mg.L^{-1}$ SA, in the extract of willow shoots collected on the 15th of January and December (8.08, 8.03, and 7.75, respectively). The cuttings soaked in the extract of willow shoots collected on 15th of September presented the least number of leaves (4.83). Further, willow extracts taken from the shoots collected on 15th of December and October exhibited the largest leaves (20.55 and 18.87 cm^2, respectively). Whereas, the smallest values of leaf area (10.07 and 10.76 cm) were recorded in the cuttings soaked in the extract of willow shoots collected on 15th of August and in control cuttings, respectively. Moreover, spectrophotometric analysis of leaf chlorophylls revealed that chlorophyll *a* (4.73 $\mu g.g^{-1}$) was the highest in the cuttings soaked in the extract of willow shoots collected on 15th of February, but it was the lowest (1.78 $\mu g.g^{-1}$) in the cuttings soaked in the extract of willow shoots collected on 15th of October. Besides, Chlorophyll *b* reached the maximum (3.09 $\mu g.g^{-1}$) in the cuttings soaked in the extract of willow shoots collected on the 15th of September, while minimum chlorophyll *b* (1.35 $\mu g.g^{-1}$) was determined in the leaves of the cuttings soaked in the extract of willow shoots collected on 15th of April. The results of survival percentage after transplanting, as indicated in table (2), also explained that there were no significant differences between the control cuttings and the cuttings that soaked in the extract of willow shoots collected in different dates. Besides, soaking the cuttings in 300 $mg.L^{-1}$ SA was the best to achieve (100%) survival after transplanting. However, the survival percentage after transplanting was the worst in the cuttings soaked in the extract of willow shoots collected on the 15th of May, June, July, August, and September, and it was more pronounced on the 15th of July and September (50%)

Table 2. Effect of 300 $mg.L^{-1}$ salicylic acid (SA), 300 $mg.L^{-1}$ indole-3-acetic acid (IAA), and willow shoot extracts collected on different dates on leaf number, leaf area, chlorophylls *a*, *b* and Survival% after transplanting of thornless blackberry cuttings.

Treatments	Leaf number	Leaf area (cm^2)	Chlorophyll *a* ($\mu g.g^{-1}$)	Chlorophyll *b* ($\mu g.g^{-1}$)	Survival% after transplanting
Control	5.83 def*	10.76 cd	2.86 f	1.83 g	83.33 ab
SA 300 $mg.L^{-1}$	8.08 a	13.78 a-d	2.22 hi	1.59 j	100.00 a
IAA 300 $mg.L^{-1}$	5.16 ef	12.34 bcd	1.95 j	1.64 i	72.22 abc
15th Jan.	8.03 a	13.63 a-d	2.58 g	1.73 h	78.33 abc
15th Feb.	6.94 a-d	15.89 a-d	4.73 a	2.87 b	80.55 abc
15th Mar.	6.47 b-e	11.18 bcd	3.32 d	1.85 g	80.55 abc
15th Apr.	6.83 a-d	16.51 a-d	2.11 i	1.35 l	70.00 abc
15th May	6.2 c-f	17.92 a-d	2.66 g	1.51 k	58.89 bc
15th Jun.	7.17 a-d	18.23 abc	4.47 b	2.72 c	66.67 bc
15th Jul.	6.4 b-e	14.86 a-d	3.4 d	2.07 e	50.00 c
15th Aug.	5.94 def	10.07 d	2.34 h	1.67 h	66.67 bc
15th Sep.	4.83 f	11.23 bcd	2.96 ef	3.09 a	50.00 c
15th Oct.	7.5 abc	18.87 ab	1.78 k	1.5 k	91.67 ab
15th Nov.	6.61 a-e	14.52 a-d	3.01 e	2.02 f	69.44 abc
15th Dec.	7.75 ab	20.55 a	3.86 c	2.37 d	83.33 ab

* Values in the same column taken the same letter were not significantly different subjected to Duncan's multiple-range test at ($p \leq 0.05$).

Figure (2) illustrates that the quantities of total phenols, total flavonoids, salicylic acid (SA), and indole3-acetic acid (IAA) were variable in willow shoots depending on the date on which the shoots were collected. In this regard, the collection of willow shoots on 15th of March and April brought about the maximal total phenols (57 and 56.27 $\mu g.mL^{-1}$) and SA (465.23 and 492.61 $\mu g.mL^{-1}$), respectively (Figure 2, A and C). Contrarily, minimal phenols (51.4, 51.7, 51.91, and 51.94 $\mu g.mL^{-1}$) were detected in the willow shoots collected on 15th of January, November, December, and August, respectively. In addition, the willow shoots collected on 15th of April had the peak value of total flavonoids (44.96 $\mu g.\ mL^{-1}$). Albeit, the least flavonoids were found in the willow shoots collected on 15th of January (29.07 $\mu g.mL^{-1}$) and 15th of August (29.18 $\mu g.mL^{-1}$). While, the collection of the willow shoots indicated that SA ratios were soaring in the willow shoots collected on 15th of April (492.61 µg.mL^{-1}) and March (465.23 µg.mL^{-1}), but the collection dates; 15th of October (197.55 µg. mL^{-1}) and January (228.57 µg.mL^{-1}) caused the lowest values. Further, IAA started to increase in the collected willow shoots from 15th of January until 15th of March (Figure 2D), and on 15th of March it was the highest (365.17 µg.mL^{-1}). Oppositely, IAA launched to decline from 15th of April to 15th of September, and it reached the minimum value (85.03 µg.mL^{-1}) on 15th of September. Once again, a slight increase in IAA was observed in the willow shoots collected on 15th of November (224.4 µg.mL^{-1}).

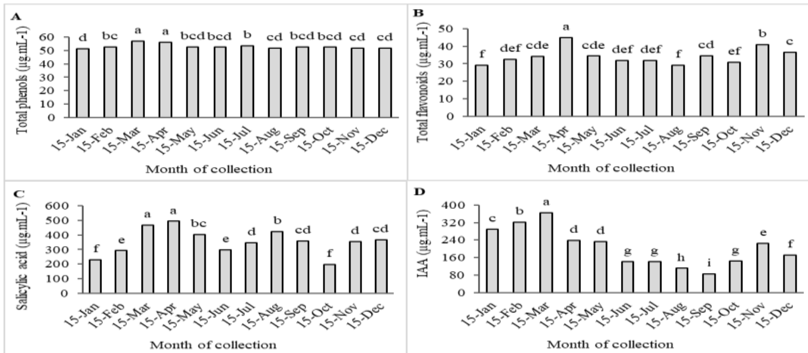

Figure 2. The concentration of total phenols (A), total flavonoids (B), salicylic acid (C), and IAA (D) in dried willow shoots collected on different dates. Columns with the same letter(s) show values which were not significantly different according to Duncan's multiple range test (P ≤ 0.05).

Pearson correlation test (P ≤ 0.05) of the means of the parameters is displayed in Figure 3, showing that callusing percentage (Cal%) was negatively linked with death percentage (D%) (P=0.03). However, it had a positive correlation with leaf area (LA) (P=0.03). Root length (RL) is positively associated with shoot length (SL), leaf number (LNo.), and leaf area (LA) (P=0.02, 0.03, and 0.03, respectively). Likewise, positive connections were found between SL and LNo. (P=0.02) as well as LA (P= 0.0001). Relationships between LNo. and LA (P=0.03), and LNo. and survival percentage after transplanting (SAT%) (P=0.01) were positive as well. In addition, chlorophyll *a* and chlorophyll *b* were positively related (P=0.0004)

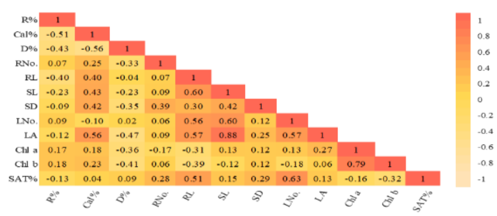

Figure 3. Interrelationships among studied parameters according to Pearson correlation test (P ≤ 0.05). Rooting percentage (R%), callusing percentage (Cal%), death percentage (D%), root number (RNo.), root length (RL), shoot length (SL), shoot diameter (SD), leaf number (LNo.), leaf area (LA), chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), and survival percentage after transplanting (SAT%)

Discussion

The proportion of phytochemicals and hormones in willow shoots which were variable along the collection dates of the shoots might have a role in the regulation of rooting, callusing, and survival percentages of the thornless blackberry cuttings (Table 1 and Figure 2). In this context, the dates on which total phenols, total flavonoids, and/or salicylic acid (SA) were high in willow shoots reduced or kept rooting to a low extent, even if IAA was the highest. Also, low total phenols, total flavonoids, and/or SA in willow shoots did not significantly induce rooting in the cuttings of thornless blackberries if IAA was also low. On the contrary, low total phenols, total flavonoids, and/or SA and high IAA appeared favorable to improve rooting. So, the extract from the willow shoots collected on 15th of January displayed the highest rooted cuttings. Meanwhile, these willow shoots had the least total phenols, total flavonoids, and the second lowest SA, but they had the third highest IAA. In parallel, the lowest rooting percentage in the current study was found in the cuttings supplied with the extracts of the willow shoots collected on 15th of August and October. The willow shoots from the 15th of August collection contained low total phenols and total flavonoids. However, the third highest SA, at the same time, the IAA level in willow shoots was the second minimum. As for the willow shoots that were collected on 15th of October, they had the lowest SA and the third lowest total flavonoids, but IAA was also still low and total phenols were fairly high as well. However, IAA was the highest in the willow shoots collected on 15th of March. However, their extract, which did not improve rooting, may be because of the highest total phenols and SA simultaneously. Evidence shows phenols may act as rooting promotors or inhibitors in cuttings [25, 26]. The type, concentration, and source of the phenols (endogenous or exogenous), as well as plant species, have a decisive effect on the adventitious root formation (ARF) in the cuttings [27]. Some of the phenols inhibit IAA oxidation but others stimulate IAA oxidation [28]. IAA is outstanding for ARF in the cuttings. In the current study, total phenols were quantified in the willow shoots which may contain rooting inhibitor phenols in the collection dates that reduce rooting. On the other hand, different conclusions have been reached with the application of exogenous SA to the cuttings of different species. Therefore, at very low concentrations of 50 and 100 µM, SA helped ARF in cucumber hypocotyl cuttings by reducing the IAA conjugate levels [29]. Whereas, [30] found in olive cuttings that exogenous SA did not trigger any root formation, even though it was concluded that exogenous SA strongly halted rooting compared to IBA and NAA. The results of the death percentage in the present study verified that the cuttings soaked in the extract of the willow shoots collected on 15th of August presented the highest death percentage which included low total phenols, total flavonoids, and IAA but high SA. Death percentages were also high among the cuttings soaked in the willow shoots from the 15th of March collection, which contained high SA, and in the cuttings soaked in 300 mg.L^{-1} SAIn spite of all of these, the phytochemicals and hormones in the willow shoots might enhance other characteristics of the thornless blackberry cuttings other than rooting, callusing, and death percentages. Thus, increasing root number due to 300 mg.L^{-1} SA, 300 mg.L^{-1} IAA, and the extract from willow shoots collected on 15th of December may belong to the fact that these treatments provided the cuttings with the necessities in a favorable concentration to rise root number in the rooted cuttings (Table 1). Reports show that SA and IAA influence some aspects of plant physiology processes about elevating root numbers, such as cell division, antioxidant activities, and oxidative stress [31]. SA and IAA may be in an optimal balance with each other and other phytochemicals in the willow shoots collected on 15th of December for the best root number. High root lengths in the cuttings treated with the extract of the willow shoots collected on the 15th of October, December, and August might be due to earlier rooting in these cuttings. Therefore, they had more time to elongate their root to the longest extent. Previous research indicated an association between earlier rooting and enhancing root length in the cuttings [32]. Besides, the phytochemicals and hormone profiles of these willow shoots were likely convenient for root elongation. In addition, the measurements revealed that the cuttings gave a better root system and concomitantly had a better shoot system (Tables 1 and 2). The thornless blackberry cuttings that possessed the best root number or root length simultaneously had the best shoot length, leaf number, and leaf area. Pearson correlation test demonstrated that a positive correlation was detected between root length and shoot length, and also between leaf number and leaf area (Figure 3). Hence, the longest root might absorb higher water and nutrients needed for better shoot growth; in turn, the shoot system does more photosynthesis and sends more photosynthates to the root required for better growth. Shukla *et al.* [33] declared that for better root and shoot growth, a balance between root and shoot ratios is essential. Moreover, it is worth mentioning that the highest survival percentage after transplanting was achieved among the cuttings which endowed with the highest numbers of roots and leaves which were clear in the cuttings treated with 300 mg.L^{-1} SA. The second higher survival percentage was recorded among the cuttings that soaked in the willow extract form the shoots collected on 15th of October which had the best root length, shoot length, and leaf area. The best root and shoot characteristics are crucial for providing the cuttings with better resources for surviving after transplanting. Survival percentage after transplanting is important because it determines the number of future new plants.

Conclusion

Based on the results obtained in this study, it can be deduced that willow shoot extracts differed in the root number, shoot length, leaf number, leaf area, and chlorophyll *a* and *b* compared to control cuttings. The extracts of the willow shoots collected on different dates significantly affected the parameters of the thornless blackberry cuttings; some dates improved some characteristics of the cuttings, and some dates decreased others, or were ineffective. Additionally, the phytochemical an d hormonal contents of the willow shoots were variable depending on the date of the collection. By the same token, it seemed that phytochemical and hormonal contents are related to the capacity of the willow shoot extracts to enhance the cutting characteristics. The best parameters measured when total phenols, total flavonoids, and/or SA were low and IAA was high, and this was prominent in the willow shoots collected on 15th of January, February, and November. However, further studies are needed to establish the outcomes of this study, particularly conducting specialized treatments to reduce the unfavorable consequences of the phenols, flavonoids, and/or SA in the willow shoots on the cuttings.

References

[1] Thimann, K. V. and Went, F. W. (1934). On the chemical nature of the root forming hormone. *Proceedings of Koninklijke Nederlandse Akademie van Wetenschappen*, 1934, 37: 456–459. https://www.cabidigitallibrary.org/doi/full/10.5555/19350700467

[2] Gaspar, T. H., Kevers, C., Faivre-Rampant, O., Crèvecoeur, M., Penel, C. L., Greppin, H. and Dommes, J. (2003). Changing concepts in plant hormone action. *In Vitro Cellular and Developmental Biology-Plant,* 39: 85-106. https://doi.org/10.1079/IVP2002393

[3] Suthar, S. (2010). Evidence of plant hormone like substances in vermiwash: An ecologically safe option of synthetic chemicals for sustainable farming. *Ecological Engineering*, 36(8): 1089-1092. https://doi.org/10.1016/j.ecoleng.2010.04.027

[4] Sulaiman, G. M., Hussien, N. N., Marzoog, T. R. and Awad, H. A. (2013). Phenolic content, antioxidant, antimicrobial and cytotoxic activities of ethanolic extract of *Salix alba*. *American Journal of Biochemistry and Biotechnology*, 9(1): 41-46. https://doi.org/10.3844/ajbbsp.2013.41.46

[5] Piątczak, E., Dybowska, M., Płuciennik, E., Kośla, K., Kolniak-Ostek, J. and Kalinowska-Lis, U. (2020). Identification and accumulation of phenolic compounds in the leaves and bark of *Salix alba* (L.) and their biological potential. *Biomolecules*, 10(10): 1-17 https://doi.org/10.3390/biom10101391

[6] Ficko, S. A. and Naeth, M. A. (2022). Influence of treatment on rooting of arctic Salix species cuttings for revegetation. *Arctic, Antarctic and Alpine Research*, 54(1): 62-77. https://doi.org/10.1080/15230430.2022.2042946

[7] Kawase, M. (1970). Root-promoting Substances in *Salix alba*. *Physiologia Plantarum*, 23(1): 159-170. https://doi.org/10.1111/j.1399-3054.1970.tb06404.x

[8] Wise, K., Gill, H. and Selby-Pham, J. (2020). Willow bark extract and the biostimulant complex Root Nectar® increase propagation efficiency in chrysanthemum and lavender cuttings. *Scientia Horticulturae*, 263: 109108. https://doi.org/10.1016/j.scienta.2019.109108

[9] Arena, M. J., Schwarz, O. J. and Witte, W. T. (1997). Experiments with locust and willow diffusates on rooting cuttings. *HortScience*, 32(4): 590. https://doi.org/10.21273/HORTSCI.32.4.590D

[10] Caplan, D., Stemeroff, J., Dixon, M. and Zheng, Y. (2018). Vegetative propagation of cannabis by stem cuttings: effects of leaf numb, cutting position, rooting hormone, and leaf tip removal. *Canadian Journal of Plant Science*, 98(5): 1126-1132. https://doi.org/10.1139/cjps-2018-0038

[11] Al-Amad, I. and Qrunfleh, M. (2016). Effect of Babylon weeping willow (Salix babylonica L.) extracts on rooting of stem cuttings of olive (*Olea europaea* L.) 'Nabali'. *Acta Horticulturae*, 1130: 391-396. https://doi.org/10.17660/ActaHortic.2016.1130.58

[12] Liu, J. and Sherif, S. M. (2019). Hormonal orchestration of bud dormancy cycle in deciduous woody perennials. *Frontiers in Plant Science*, 10, 1136. https://doi.org/10.3389/fpls.2019.01136

[13] Tienaho, J., Reshamwala, D., Sarjala, T., Kilpeläinen, P., Liimatainen, J., Dou, J., Viherä-Aarino, A., Linnakoski, R., Marjomäki, V. and Jyske, T. (2021). *Salix* spp. Bark Hot Water Extracts Show Antiviral, Antibacterial, and Antioxidant Activities—The Bioactive Properties of 16 Clones. *Frontiers in Bioengineering and Biotechnology*, 9: 1-19 https://doi.org/10.3389/fbioe.2021.797939

[14] Förster, N., Ulrichs, C., Zander, M., Kätzel, R. and Mewis, I. (2008). Influence of the Season on the Salicylate and Phenolic Glycoside Contents in the Bark of *Salix Daphnoides*, *Salix Pentandra* and *Salix Purpurea*. *Journal of Applied Botany and Food Quality*, 82(99): 99–102. https://ojs.openagrar.de/index.php/JABFQ/article/view/2086

[15] Dziedzic, E. and Jagla, J. (2012). Micropropagation of *Rubus* and *Ribes* spp. In: Lambardi, M., Ozudogru, E. A., Jain, S. M. (eds.) Protocols for micropropagation of selected economically-important horticultural plants. Humana Press, Totowa, NJ pp. 149–160. https://doi.org/10.1007/978-1-62703-074-8_11

[16] Reed, B., Poothong, S. and Hall, H. K. (2017). Propagation of blackberries and related Rubus species. In: Hall HK, Funt RC (ed) Blackberries and their hybrids. Wallingford, UK, CABI. pp.101-112. https://doi.org/10.1079/9781780646688.0101

[17] Coyner, M.bA., Skirvin, R. M., Norton, M. A. and Otterbacher, A. G. (2005). Thornlessness in blackberries: A review. *Small Fruits Review*, 4(2): 83-106. https://doi.org/10.1300/J301v04n02_09

[18] Takeda, F., Tworkoski, T., Finn, C. E. and Boyd, C. C. (2011). Blackberry propagation by non-leafy floricane cuttings. *HortTechnology*, 21(2): 236-239. https://doi.org/10.21273/HORTTECH.21.2.236

[19] Debner, A. R., Hatterman-Valenti, H. and Takeda, F. (2019). Blackberry propagation limitations when using floricane cuttings. *HortTechno*, 29(3): 276-282. https://doi.org/10.21273/HORTTECH04266-18

[20] Tahir, N. A., Rasul, K. S., Lateef, D. D. and Grundler, F. M. (2022). Effects of oak leaf extract, biofertilizer, and soil containing oak leaf powder on tomato growth and biochemical characteristics under water stress conditions. *Agriculture*, 12(12): 2082. https://doi.org/10.3390/agriculture12122082

[21] Gordon, S. A. and Weber, R. P. (1951). Colorimetric estimation of indoleacetic acid. *Plant Physiology*, 26(1): 192-195 https://doi.org/10.1104/pp.26.1.192

[22] Husen, A., Iqbal, M., Siddiqui, S. N., Sohrab, S. S. and Masresha, G. (2015). Effect of indole-3-butyric acid on clonal propagation of mulberry (*Morus alba* L.) stem cuttings: rooting and associated biochemical changes. *Proceedings of the National Academy of Sciences, India Section B-Biological Sciences*, 87(1): 161-166. https://doi.org/10.1007/s40011-015-0597-7

[23] Warrier, R. R., Paul, M. and Vineetha, M. V. (2013). Estimation of salicylic acid in Eucalyptus leaves using spectrophotometric methods. *Genetics and Plant Physiology*, 3(1-2): 90-97. http://www.bio21.bas.bg/ippg/bg/wp-content/uploads/2014/03/GPP_3_1-2_90-97.pdf

[24] Su, S., Zhou, Y., Qin, J. G., Yao, W. and Ma, Z. (2010). Optimization of the method for chlorophyll extraction in aquatic plants. Journal of Freshwater Ecology, 25(4): 531-538. https://doi.org/10.1080/02705060.2010.9664402

[25] Štefančič, M., Štampar, F., Veberič, R. and Osterc, G. (2007). The levels of IAA, IAAsp and some phenolics in cherry rootstock 'Gisela 5' leafy cuttings pretreated with IAA and IBA. *Scientia Horticulturae*, 112(4): 399-405. https://doi.org/10.1016/j.scienta.2007.01.004

[26] Trobec, M., Štampar, F., Veberič, R. and Osterc, G. (2005). Fluctuations of different endogenous phenolic compounds and cinnamic acid in the frst days of the rooting process of cherry rootstock 'GiSelA 5' leafy cuttings. *Journal of Plant Physiology*, 162(5): 589–597. https://doi.org/10.1016/j.jplph.2004.10.009

[27] Denaxa, N. K., Tsafouros, A. and Roussos, P. A. (2023). Role of phenolic compounds in adventitious root formation. In: Husen A (ed) Environmental, physiological and chemical controls of adventitious rooting in cuttings. Academic Press, pp. 251-288. https://doi.org/10.1016/B978-0-323-90636-4.00013-1

[28] Denaxa, N. K., Roussos, P. A. and Vemmos, S. N. (2020). Assigning a role to the endogenous phenolic compounds on adventitious root formation of olive stem cuttings. *Journal of Plant Growth Regulation*, 39: 411-421. https://doi.org/10.1007/s00344-019-09991-0

[29] Dong, C. J., Liu, X. Y., Xie, L. L., Wang, L. L. and Shang, Q. M. (2020). Salicylic acid regulates adventitious root formation via competitive inhibition of the auxin conjugation enzyme CsGH3. 5 in cucumber hypocotyls. *Planta*, 252(75): 1-15. https://doi.org/10.1007/s00425-020-03467-2

[30] İsfendiyaroğlu, M. and Oezeker, E. (2008). Rooting of Olea europaea "Domat" cuttings by auxin and salicylic acid treatments. *Pakistan Journal of Botany*, 40(3): 1135-1141. https://www.pakbs.org/pjbot/PDFs/40(3)/PJB40(3)1135.pdf

[31] Bagautdinova, Z. Z., Omelyanchuk, N., Tyapkin, A. V., Kovrizhnykh, V. V., Lavrekha, V. V. and Zemlyanskaya, E. V. (2022). Salicylic acid in root growth and development. *International Journal of Molcular Sciences*, 23(4): 1-26. https://doi.org/10.3390/ijms23042228

[32] Saranga, J. and Cameron, R. (2007). Adventitious root formation in *Anacardium occidentale* L. in response to phytohormones and removal of roots. *Scientia Horticulturae*, 111(2): 164-172. https://doi.org/10.1016/j.scienta.2006.10.010

[33] Shukla, H. S., Tripathi, V. K., Awasthi, R. D. and Tripathi, A. K. (2010). Effect of IBA, PHB and boron on rooting and shoot growth of hardwood stem cuttings of Peach. *International Journal of Applied Agricultural Research*, 5(4): 467-473. https://www.ripublication.com/Volume/ijaarv5n4.htm

تجذير اقلام توت العليق اللاشوكي المستحثة بمستخلص الصفصاف الأبيض
المجمعة في مواعد مختلفة (*Salix alba* L.)

كوجر عمر صالح[1] **ئارام اكرم محمد[1]** **ابراهيم معروف نوري[1]**

[1] قسم البستنة، كلية علوم الهندسة الزراعية، جامعة السليمانية، السليمانية، إقليم كردستان، العراق..

لخلاصة

يحتوي المستخلص المائي لنبات *Salix spp* على العديد من المركبات التي قد تعمل كعوامل تعزيز الجذور في العقل. *S. alba* هي شجرة متساقطة الأوراق التي تحتوي على العديد من المواد الكيميائية النباتية والهرمونات المتغايرة تختلف على مدار العام. لذلك في هذه الدراسة تم جمع الافروع *S. alba* بعمر سنة وسنتين في الخامس عشر من كل شهر في عام 2022، واستخلاصها في 2% إيثانول بمعدل 9 غ/لتر ووضعها في حمام مائي بدرجة حرارة 35 °*C*، ثم عوملت بها اقلام توت العليق اللاشوكي لمدة 1.5 ساعة. أوضحت النتائج أن أعلى نسبة تجذير (67.66%) تم الحصول عليها في العقل المغمورة بمستخلص افرع الصفصاف المجمعة بموعد 15 كانون الثانى التي لم تختلف معنوياً عن عقل المقارنة، لكنها كانت مختلفة عن العقل المغمورة في مستخلص افرع الصفصاف التي تم جمعها في 15 أب وتشرين الاول بنسبة (33.33%). كانت غالبية السمات الخضرية والجذرية الأخرى عالية في العقل المغمورة في مستخلص افرع الصفصاف التي تم جمعها في 15 ديسمبر. احتوت افرع الصفصاف التي تم جمعها في 15 كانون الثانى على أقل إجمالي للفينولات (51.4 ميكروغرام/مل) وإجمالي الفلافونويدات (29.07 ميكروغرام/مل). علاوة على ذلك، تم تسجيل أعلى إجمالي للفينولات (57 ميكروغرام/مل)، *SA* (492.61 ميكروغرام/مل)، و*IAA* (365.17 ميكروغرام/مل) في افرع الصفصاف التي تم جمعها في 15 ايار، وكان إجمالي مركبات الفلافونويد في أعلى مستواها (44.96 ميكروغرام/مل) في افرع الصفصاف التي تم جمعها في 15 نيسان. بشكل عام، بناءً على نسبة التجذير، يُنصح بجمع افرع الصفصاف في 15 كانون الثانى وشباط لاستخراجها وتطبيقها على اقلام توت العليق اللاشوكي.

الكلمات المفتاحية : الفلافونويدات، *IAA*، الفينولات، *SA* ، التجذير.