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

Kocher Omer Salih1  Aram Akram Mohammed1  Ibrahim Maaroof Noori1

1 Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaimani, Kurdistan Region, Iraq.

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, phytochemicals and hormones in deciduous trees are variable 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 applied to thornless blackberry cuttings for 1.5 h. The results explained that the highest rooting percentage (66.66%) was obtained in the cuttings soaked in the extract of willow shoots collected on 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 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 15th of December. The willow shoots collected on 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), SA (492.61 µg. mL⁻¹), and IAA (365.17 µg. mL⁻¹) were recorded in the willow shoots collected on 15th of March, and total flavonoids were the highest (44.96 µg. mL⁻¹) in the willow shoots collected on 15th of April. Generally, based on rooting percentage, it is advisable to collect willow shoots on 15th of January and February for extract and application to the thornless blackberry cuttings.

Keywords: flavonoids, IAA, phenols, rooting, SA.

Citation: Salih, K., Mohammed, A., & Noori, I. (2024). Rooting of thornless blackberry cuttings as induced by the extract of white willow (Salix alba L.) shoots collected in different times. Kirkuk University Journal for Agricultural Sciences, 15(1), - doi: 10.58928/kujas.2024.147482.1138

Correspondence Author: Kocher Omer Salih_kocher.salih@univsul.edu.iq

Copyright: This is an open access article distributed under the terms of the creative common’s license, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.
**Introduction**

Since the findings by [1], it has been approved that auxin indole acetic acid (IAA) has a crucial role 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 which is 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 [2]. Therefore, many attempts have been exerted to find natural sources of auxins and other substances that promote root formation in cuttings with lower cost and lesser harm to humans and the ecosystem [3]. Among the most popular natural extracts that are 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 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 different results when applying 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 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 didn’t give a favorable rooting in stem cuttings of cannabis [10]. Besides, [11] observed that 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]. [13] reported that seasonal and environmental factors caused changes in phytochemical contents in the bark of Salix spp. Besides, [14] referred that during the vegetative season, precisely from March to July, secondary metabolite numbers 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]. 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 does 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 floricanne cuttings of blackberries is limited and variable. Nonetheless, cultivar, the time of taking cuttings, using auxins, and the cuttings atmosphere have a decisive role in the successful rooting of blackberry cuttings [18, 19]. In light of the above-mentioned reasons, in this study, willow shoots were collected at different times and extracted, and then applied to the cuttings of thornless blackberry in order 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 and extract of willow shoots
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 the time of extraction.

Quantification of total phenols and flavonoids in willow shoots
The dried willow shoots were collected at different times ground in a blender and used for analysis of 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 change in the methods described by [21, 22]. So, 1.5 g of dried and well-ground shoots of willow were weighted and mixed well with 10 mL of salkowiski reagent, shaken thoroughly, and left for 1 hour. After that, the mixture was filtered through filter paper, and the absorbance was read at 530 nm. The concentration of IAA was found using the IAA standard curve and represented as µg. mL⁻¹.

Determination of salicylic acid in willow shoots
Salicylic acid was determined using a spectrophotometer as described by [23], with a slight change. The samples were prepared by weighing 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 FeCl3 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 µg. mL⁻¹.

Preparation of willow shoot extracts
The extraction was initiated by grinding the dried willow shoots in a blender. The ground willow shoots which were taken from each collecting time separately weighed at (9 g) and extracted in (1L) of 2% ethanol placed in a water bath at 35 °C for 3 hours, and refrigerated for 24h. After that, the extracts were filtered through filter paper, 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 12 cm long (containing at least three buds) and 4-7.5 mm diameter. After shipping to the laboratory, they were soaked in 54% topsin fungicide at 1.5 ml/L for 30 min. The cuttings, after the surface dry, 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 hours. The treated cuttings were stuck in sand media prepared in black plastic pots, 7 cuttings per pot, and they were laid out in randomized complete block design (RCBD) with three replications inside a high tunnel. The average minimum and maximum temperatures inside the high tunnel are shown in Figure (1).
Figure 1. Average minimum and maximum temperature inside the high tunnel, from February 12 till May 3, 2023.

Statistical analysis
The data were measured 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, chlorophyll an and b in the leaves, and survival percentage after transplanting. To calculate the survival percentage after transplanting, 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 temperature inside the lath house was between (15.7- 33.4 °C). Chlorophyll an 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 test at (p ≤ 0.05) for comparison of the means.

Results
Analysis of the data showed that extract of willow shoots that were collected on different dates gave different rooting%, callusing%, death%, and root lengths (Table 1), but no collection dates of willow shoots showed significant differences in rooting%, callusing%, death%, and root length related to control cuttings. The highest rooting percentage (66.66%) was detected in the cuttings treated with the extract of willow shoots collected on 15th of January followed by 15th of November (57.14%), and 15th of February (52.38%). In contrast, the lowest rooting percentage (33.33%) was in the cuttings treated with the extract of willow shoots collected on 15th of August and October accompanied by (38.09%) on 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 15th of December, but it was the least (14.28%) in the treated cuttings with the extract of willow shoots collected on 15th of January. No dead cuttings were observed in the ones treated with the extract of willow shoots collected on 15th of December. Also, the willow shoot extract from the collection date of 15th of November showed the lowest death (4.76%) of the thornless blackberry cuttings. While, the extract from the willow shoots collected on 15th of August resulted in the maximum number of dead cuttings (38.09%), followed by 15th of March (23.81%), and treatment with 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 from the willow shoots collected on 15th of September and February caused the shortest roots (13.46 and 13.79 cm, respectively).
The data in the same table confirmed that soaking thornless blackberry cuttings in extracts
of willow shoots which were collected on 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 15\(^{th}\) 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 15\(^{th}\) of March and July gave the minimum number of roots (4.33 and 4.67, respectively). Similarly, the collection of willow shoots on 15\(^{th}\) of December and the extract of them 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 15\(^{th}\) 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.

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 No., root length, shoot length, and shoot diameter of thornless blackberry cuttings.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Rooting%</th>
<th>Callusing%</th>
<th>Death%</th>
<th>Root length (cm)</th>
<th>Root No.</th>
<th>Shoot length (cm)</th>
<th>Shoot diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42.85 ab</td>
<td>42.85 ab</td>
<td>14.28 ab</td>
<td>17.55 ab</td>
<td>6.93 cd</td>
<td>2.59 cd</td>
<td>1.98 a</td>
</tr>
<tr>
<td>SA 300 mg.L(^{-1})</td>
<td>42.85 ab</td>
<td>33.33 ab</td>
<td>23.80 ab</td>
<td>17.63 ab</td>
<td>16.17 a</td>
<td>4.5 a-d</td>
<td>1.91 a</td>
</tr>
<tr>
<td>IAA 300 mg.L(^{-1})</td>
<td>47.62 ab</td>
<td>38.09 ab</td>
<td>14.28 ab</td>
<td>15.19 ab</td>
<td>14.67 a</td>
<td>2.61 cd</td>
<td>1.85 a</td>
</tr>
<tr>
<td>15-Jan</td>
<td>66.66 a</td>
<td>14.28 b</td>
<td>19.04 ab</td>
<td>18.07 ab</td>
<td>7.5 bcd</td>
<td>3.43 bcd</td>
<td>1.74 a</td>
</tr>
<tr>
<td>15-Feb</td>
<td>52.38 ab</td>
<td>38.09 ab</td>
<td>9.52 ab</td>
<td>13.79 b</td>
<td>7.44 bcd</td>
<td>3.92 a-d</td>
<td>1.93 a</td>
</tr>
<tr>
<td>15-Mar</td>
<td>42.85 ab</td>
<td>33.33 ab</td>
<td>23.81 ab</td>
<td>15.11 ab</td>
<td>4.33 d</td>
<td>2.52 cd</td>
<td>1.66 a</td>
</tr>
<tr>
<td>15-Apr</td>
<td>38.09 b</td>
<td>42.85 ab</td>
<td>19.04 ab</td>
<td>20.96 ab</td>
<td>6.9 cd</td>
<td>3.88 a-d</td>
<td>1.7 a</td>
</tr>
<tr>
<td>15-May</td>
<td>47.61 ab</td>
<td>42.85 ab</td>
<td>9.52 ab</td>
<td>15.31 ab</td>
<td>8.53 bc</td>
<td>5.65 ab</td>
<td>1.87 a</td>
</tr>
<tr>
<td>15-Jun</td>
<td>38.09 b</td>
<td>42.85 ab</td>
<td>19.05 ab</td>
<td>17.03 ab</td>
<td>7.62 bcd</td>
<td>4.97 abc</td>
<td>1.88 a</td>
</tr>
<tr>
<td>15-Jul</td>
<td>47.61 ab</td>
<td>33.3 ab</td>
<td>18.95 ab</td>
<td>15.34 ab</td>
<td>4.67 d</td>
<td>4.79 a-d</td>
<td>1.77 a</td>
</tr>
<tr>
<td>15-Aug</td>
<td>33.33 b</td>
<td>28.57 ab</td>
<td>38.09 a</td>
<td>17.51 ab</td>
<td>7.75 bcd</td>
<td>3.72 a-d</td>
<td>1.94 a</td>
</tr>
<tr>
<td>15-Sep</td>
<td>47.62 ab</td>
<td>42.85 ab</td>
<td>9.52 ab</td>
<td>13.46 b</td>
<td>11.08 b</td>
<td>2.16 d</td>
<td>1.79 a</td>
</tr>
<tr>
<td>15-Oct</td>
<td>33.33 b</td>
<td>47.61 ab</td>
<td>19.04 ab</td>
<td>25.89 a</td>
<td>6.37 cd</td>
<td>5.9 ab</td>
<td>1.95 a</td>
</tr>
<tr>
<td>15-Nov</td>
<td>57.14 ab</td>
<td>38.09 ab</td>
<td>4.76 b</td>
<td>17.17 ab</td>
<td>9.87 bc</td>
<td>4.35 a-d</td>
<td>2.07 a</td>
</tr>
<tr>
<td>15-Dec</td>
<td>42.85 ab</td>
<td>57.14 a</td>
<td>0 b</td>
<td>23.78 ab</td>
<td>14.8 a</td>
<td>6.23 a</td>
<td>2.02 a</td>
</tr>
</tbody>
</table>

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

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 they were compared with control cuttings (Table 2). Leaf number was plethora in the cuttings soaked in the 300 mg. L\(^{-1}\) SA, in the extract of willow shoots collected on 15\(^{th}\) of January and December (8.08, 8.03, and 7.75, respectively). The cuttings soaked in the extract of willow shoots collected on 15\(^{th}\) of September presented the least number of leaves (4.83). Further, willow extracts from the shoots collected on 15\(^{th}\) of December and October exhibited the largest leaf (20.55 and 18.87 cm\(^2\), respectively). Whereas, the smallest leaf areas (10.07 and 10.76 cm\(^2\)) were recorded in the cuttings soaked in the extract of willow shoots collected on 15\(^{th}\) of August and in control cuttings, respectively. Moreover, spectrophotometric analysis of leaf chlorophylls revealed that the ratio of
chlorophyll a (4.73 µg 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 µg g⁻¹) in the soaked cuttings in the extract of willow shoots collected on 15th of October. Besides, Chlorophyll b reached the maximum (3.09 µg g⁻¹) in the cuttings soaked in the extract of willow shoots collected on the 15th of September, while minimum chlorophyll b (1.35 µg 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 in table (2) also explained that there were no significant differences between the control cuttings and the cuttings were soaked in the extract of willow shoots from different date collections. Accordingly, 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 of salicylic acid (SA), 300 mg L⁻¹ indole-3-acetic acid (IAA), and willow shoot extracts collected on different dates on leaf No., leaf area, chlorophyll a, chlorophyll b, and Survival% after transplanting of thornless blackberry cuttings.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Leaf No.</th>
<th>Leaf area (cm²)</th>
<th>Chlorophyll a (µg.g⁻¹)</th>
<th>Chlorophyll b (µg.g⁻¹)</th>
<th>Survival% after transplanting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.83 def</td>
<td>10.76 cd</td>
<td>2.86 f</td>
<td>1.83 g</td>
<td>83.33 ab</td>
</tr>
<tr>
<td>SA 300 mg.L⁻¹</td>
<td>8.08 a</td>
<td>13.78 a-d</td>
<td>2.22 hi</td>
<td>1.59 j</td>
<td>100 a</td>
</tr>
<tr>
<td>IAA 300 mg.L⁻¹</td>
<td>5.16 ef</td>
<td>12.34 bcd</td>
<td>1.95 j</td>
<td>1.64 i</td>
<td>72.22 abc</td>
</tr>
<tr>
<td>15-Jan</td>
<td>8.03 a</td>
<td>13.63 a-d</td>
<td>2.58 g</td>
<td>1.73 h</td>
<td>78.33 abc</td>
</tr>
<tr>
<td>15-Feb</td>
<td>6.94 a-d</td>
<td>15.89 a-d</td>
<td>4.73 a</td>
<td>2.87 b</td>
<td>80.55 abc</td>
</tr>
<tr>
<td>15-Mar</td>
<td>6.47 b-e</td>
<td>11.18 bcd</td>
<td>3.32 d</td>
<td>1.85 g</td>
<td>80.55 abc</td>
</tr>
<tr>
<td>15-Apr</td>
<td>6.83 a-d</td>
<td>16.51 a-d</td>
<td>2.11 i</td>
<td>1.35 l</td>
<td>70 abc</td>
</tr>
<tr>
<td>15-May</td>
<td>6.2 c-f</td>
<td>17.92 a-d</td>
<td>2.66 g</td>
<td>1.51 k</td>
<td>58.89 bc</td>
</tr>
<tr>
<td>15-Jun</td>
<td>7.17 a-d</td>
<td>18.23 abc</td>
<td>4.47 b</td>
<td>2.72 c</td>
<td>66.67 bc</td>
</tr>
<tr>
<td>15-Jul</td>
<td>6.4 b-e</td>
<td>14.86 a-d</td>
<td>3.4 d</td>
<td>2.07 e</td>
<td>50 c</td>
</tr>
<tr>
<td>15-Aug</td>
<td>5.94 def</td>
<td>10.07 d</td>
<td>2.34 h</td>
<td>1.67 h</td>
<td>66.67 bc</td>
</tr>
<tr>
<td>15-Sep</td>
<td>4.83 f</td>
<td>11.23 bcd</td>
<td>2.96 ef</td>
<td>3.09 a</td>
<td>50 c</td>
</tr>
<tr>
<td>15-Oct</td>
<td>7.5 abc</td>
<td>18.87 ab</td>
<td>1.78 k</td>
<td>1.5 k</td>
<td>91.67 ab</td>
</tr>
<tr>
<td>15-Nov</td>
<td>6.61 a-e</td>
<td>14.52 a-d</td>
<td>3.01 e</td>
<td>2.02 f</td>
<td>69.44 abc</td>
</tr>
<tr>
<td>15-Dec</td>
<td>7.75 ab</td>
<td>20.55 a</td>
<td>3.86 c</td>
<td>2.37 d</td>
<td>83.33 ab</td>
</tr>
</tbody>
</table>

Values in the same column taken the same letter were not significantly different subjected to Duncan’s multiple-range test at (p ≤ 0.05).

Figure (2) illustrates that the quantity of total phenols, total flavonoids, salicylic acid (SA), and indole-3-acetic acid (IAA) was apparently 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 µg mL⁻¹) and SA (465.23 and 492.61 µg mL⁻¹), respectively (Figure 2A and 2C). Contrarily, minimal phenols (51.4, 51.7, 51.91, and 51.94 µg 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 µg. mL⁻¹). Albeit, the least flavonoids were found in the willow shoots which were collected on 15th of January (29.07 µg. mL⁻¹) and 15th of August (29.18 µg. 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 on 15th of October (197.55 µg. mL⁻¹) and January (228.57 µg. mL⁻¹) were 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 were collected on different dates. Values in the same column taken the same letter were not significantly different subjected to Duncan’s multiple-range test at (p ≤ 0.05).

Pearson correlation test (p ≤ 0.05) of the means of the parameters was displayed in figure (3) showed that callusing percentage (Cal%) was negatively linked with death percentage (D%) (p=0.03), but 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 (Chl a) and chlorophyll b (Chl b) positively related (p=0.0004).
Interrelationship among studied parameters according to Pearson correlation test ($p \leq 0.05$). 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 death 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 didn’t 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 to be favorable to improve rooting. So, the extract from the willow shoots collected on 15th of January displayed the highest rooted cuttings, at the meantime 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, but the third highest SA, at the same time IAA in these willow shoots was the second minimum level. 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, but their extract didn’t improve rooting may be because of the highest total phenols and SA at the same time. There is a body of evidence that phenols may act as rooting promotors or inhibitors in cuttings [25, 26]. The type, concentration, and source of the phenols (endogenous or exogenous), and 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 they concluded that exogenous SA strongly halted rooting in comparison 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 SA.

In spite of all 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 that these treatments provided the cuttings with the necessities in a favorable concentration to rise root number in the rooted cuttings (Table 1). There are reports that SA and IAA influence some aspects of plant physiology processes in relation to 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 as well. High root lengths in the cuttings treated with the extract of the willow shoots collected on 15th of October, December, and August might be
due to earlier rooting happening 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, 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 did more photosynthesis and sent more photosynthates to the root required for better growth. [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 were endowed with the best root number and leaf number which was clear in the cuttings treated with 300 mg. L⁻¹ SA, and 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 required 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 made differences just 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 and 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 were 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


https://doi.org/10.1007/s00344-019-09991-0
https://doi.org/10.1007/s00344-020-03467-2
https://www.pakbs.org/pjbot/PDFs/40(3)/PJB40(3)1135.pdf
https://doi.org/10.1016/j.scienta.2006.10.010
https://www.ripublication.com/Volume/iJaarv5n4.htm
تجذير أقلاع توت العليق الاشوكي المستحثة بمستخلص الصفصاف الأبيض
المجموعة في مواعد مختلفة

(\textit{Salix alba} L.)

أبراهيم معروف نوري 1
نارم اكرم محمد 1
كوچر عمر صالح 1

brahim.nwri@univsul.edu.iq  aram.hamarashed@univsul.edu.iq  kocher.salih@univsul.edu.iq

قسم الريف، كلية فنادق الاراضي، جامعة السليمانية، السليمانية، كردستان، العراق.


الخلاصة

\textit{S. alba} هو شجرة شجرة متساقطة الأوراق، والمواد الكيميائية النباتية والهرمونات في الأشجار المتساقطة تختلف على مدار العام. لذلك في هذه الدراسة تم جمع الأقلاع 
\textit{S. alba} بلغ عمراً وسنتين في الخامس من كل شهر في عام 2022، واستخلاصها في 2% إيثانول بمعدل 9 جم/لتر ووضعها في حمام مائي بدرجة حرارة 35 °C، ثم مستخدم

الأقلاع التوت العليق الاشوكي لمدة 1.5 ساعة. أوضحت النتائج أن أعلى نسبة تجذير (66.66%) تم الحصول عليها في العقل المغمورة بمستخلص الاقلاع الصفصاف المجمعة في مواعيد 15 كانون الثاني لم تختلف معنوى عن العقل المقارنة، لكنها كانت مختلفة عن العقل المغمورة في مستخلص الأقلاع الصفصاف المجمعة في مواعيد 15 كانون الثاني. لم تختلف معنايا عن العقل المقارنة، لكنها كانت مختلفة عن العقل المغمورة في مستخلص الأقلاع الصفصاف والتي تم جمعها في 15 أيار وتشرين الأول بنسبة (33.33%). كانت غالبية سمات الحضاري والجذري الأخرى عالية في العقل المغمورة في مستخلص الأقلاع الصفصاف التي تم جمعها في 15 كانون الثاني على أقل إجمالي للفينولات (51.4 ميكروجرام/ملم) وإجمالي الفلافونويدات (29.07 ميكروجرام/ملم). علاوة على ذلك، تم تسجيل أعلى إجمالي للفينولات (57 ميكروجرام/ملم)، وIAA (492.61 ميكروجرام/ملم)، SA (492.61 ميكروجرام/ملم) في الأقلاع الصفصاف التي تم جمعها في 15 أيار، وكان إجمالي مركبات الفلافونويد أعلى مستوى (44.96 ميكروجرام/ملم) في الأقلاع الصفصاف التي تم جمعها في 15 نيسان. بشكل عام، بناءً على نسبة التجذير، ينصح بجمع لأقلاع الصفصاف في 15 كانون الثاني وشباط لاستخراجها وتطبيقها على

الكلمات المفتاحية: الفلافونويدات، الفينولات، التاجير، SA