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Original article

In vitro propagation of *Salix babylonica* L. from nodal segments

Propagación *in vitro* de *Salix babylonica* L. a partir de segmentos nodales

Propagação in vitro de *Salix babylonica L.* a partir de segmentos nodais

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ABSTRACT

The aim of this work was to establish a method for the *in vitro* micropropagation of *Salix babylonica L.* Three treatments were used in the disinfection experiment: with 1 % sodium hypochlorite, 0.1 % mercury bichloride and a double disinfection with both disinfecting agents. With this last treatment 71 % of nodal segments were disinfected. In the in vitro establishment experiment, 6-BAP 2 mg L^{-1} was used alone and combined with gibberellic acid (5 mg L^{-1}), AIA (1 mg L^{-1}) and ANA (1 mg L^{-1}). The best response was achieved with the use of BAP alone with 31.6 % establishment and an average length of nodal segments of 6.47 mm. For the multiplication experiment a comparison of Palomos-Rios, Chung and Carrasco and MMULT-1 culture media was made. The multiplication of the nodal segments was higher in the Palomo-Rios culture medium with 100 % sprouting and a number of nodal segments of 6.42. Different concentrations of AIB (0.05 and 0.25 mg L^{-1}) and a treatment without growth regulator were used in the rooting stage. The highest rooting percentage was obtained in the basal culture medium without AIB with 91.6 % response.

Keywords: *Salix babylonica; Micropropagation*; *In vitro* culture; Growth regulators.

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RESUMEN

El objetivo de este trabajo fue establecer una vía para la micropropagación *in vitro* de *Salix babylonica* L. En el experimento de desinfección se emplearon tres tratamientos: con hipoclorito de sodio al 1 %, bicloruro de mercurio al 0,1 % y una doble desinfección con ambos agentes desinfectantes. Con este último tratamiento se obtuvo un 71 % de segmentos nodales desinfectados. En el experimento del establecimiento *in vitro* se empleó el 6-BAP 2 mg L^{-1} solo y combinado con ácido giberélico (5 mg L^{-1}), AIA (1 mg L^{-1}) y ANA (1 mg L^{-1}). La mejor respuesta se logró con el uso de BAP solo con un 31,6 % de establecimiento y una longitud promedio de los segmentos nodales de 6,47 mm. Para el experimento de multiplicación se realizó una comparación de los medios de cultivos Palomos-Ríos, Chung y Carrasco y MMULT-1. La multiplicación de los segmentos nodales fue superior en el medio de cultivo Palomo-Ríos con un 100 % de brotación y un número de segmentos nodales de 6,42. En la etapa de enraizamiento se utilizaron diferentes concentraciones de AIB (0,05 y 0,25 mg L^{-1}) y un tratamiento sin regulador de crecimiento. El mayor porcentaje de enraizamiento se obtuvo en el medio de cultivo basal sin AIB con un 91,6 % de respuesta.

Palabras clave: *Salix babylonica*; Micropropagación; Cultivo *in vitro*; Reguladores del crecimiento.

RESUMO

O objectivo deste trabalho era estabelecer um caminho para a micro propagação in vitro da *Salix babylonica L*. Foram utilizados três tratamentos na experiência de desinfecção: com 1 % de hipoclorito de sódio, 0,1 % de bi cloreto de mercúrio e uma desinfecção dupla com ambos os agentes desinfetantes. Com este último tratamento, 71 % dos segmentos nodais foram desinfetados. Na experiência de estabelecimento in vitro, 6-BAP 2 mg L^{-1} foi utilizado sozinho e combinado com ácido giberélico (5 mg L^{-1}), AIA (1 mg L^{-1}) e ANA (1 mg L^{-1}). A melhor resposta foi obtida apenas com a utilização de BAP com 31,6 % de estabelecimento e um comprimento médio de segmentos nodais de 6,47 mm. Para a experiência de multiplicação foi feita uma comparação dos meios de cultivo Palomos-Rios, Chung e Carrasco assim como MMULT-1. A multiplicação dos segmentos nodais foi maior no meio de cultivo Palomo-Rios com 100 % de brotação e um número de segmentos nodais de 6,42. Diferentes concentrações de AIB (0,05 e 0,25 mg L^{-1}) e um tratamento sem regulador de crescimento foram utilizados na fase de enraizamento. A maior percentagem de enraizamento foi obtida no meio de cultura basal sem AIB com 91,6 % de resposta.

Palavras-chave: *Salix babylonica*; Micro propagação; Cultura *in vitro*; Reguladores de crescimento.

INTRODUCTION

Native to temperate and subtropical zones, *Salicaceae* trees and shrubs, including poplars (*Populus spp*.) and willows (*Salix spp*.) are fast growing and easily vegetated. Many of the species are adapted to a wide range of climate and soil conditions, from the heat of the Chinese desert to the cold and wind of the South American Andes. They are easy to grow and are an important component of agroforestry systems, often for small-scale farmers. They supply a wide range of wood and non-wood products and services (Ball *et al.,* 2005).

The species *Salix babylonica* has an ornamental use. However, it is also used in the phytoremediation of soils affected by hazardous substances (Ghasemi and Moktari, 2019), considered as an alternative for the elimination of sodium cyanide. This species is used for the production of renewable energy. In this regard, Doffo *et al*., (2017) reported the diversification of renewable sources such as forest biomass, produced by *Salix*; for this purpose, they considered that water availability is the main factor that determines the yield in dry biomass, while genotype and plantation density do not have a significant effect on the mentioned variable.

In addition, this species is known to be used for obtaining acetylsalicylic acid or aspirin. However, new applications in the field of medicine and particularly its antimicrobial effects have been reported recently. Wahab *et al., (2018)* determined that methanolic extracts of leaves and bark of *S. babylonica* possess antibacterial activity against *Pseudomona aeruginosa, Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus.* Also, González *et al., (2019)* indicated that compounds isolated from the hydroalcoholic extract *S. babylonica* could be a natural and functional alternative for the treatment of diseases caused by *Escherichia coli, Staphylococcus aureus and Listeria monocytogenes.*

In other studies, Rivero *et al.*, (2019) showed that oral administration of hydroalcoholic extract of *S. babylonica* could be a natural alternative for the control of coccidiosis in rabbit production.

The application of biotechnological methods in different *Salix species* has focused mainly on micropropagation (Naujok, 2007; Chornobrov, 2015; López *et al*., 2016; Grendysz *et al*., 2017) to respond to the needs for accelerated propagation of different species and genotypes, the rooting of recalcitrant varieties and as a basis for the application of other in vitro cultivation techniques.

Skálová *et al.,* (2012) developed micropropagation from apical shoot and nodal segments of common and endangered willow (*Salix sp.*) species, which can help reintroduce native genotypes into their natural sites. In addition, they cultivated isolated anthers in selected media and achieved the formation of *S. caprea* and *S. viminalis*.

Imran *et al.,* (2018) produced non-embryogenic synthetic seeds; by encapsulating nodal segments of *S. tetrasperma* in a calcium hydrogel culture medium, they observed that the encapsulated buds survived cold storage at 4°C for up to 8 weeks and achieved a 71 % conversion rate to seedlings.

Biotechnological methods have also been used for cryopreservation of germplasm (Bonnart *et al*., 2014) and obtaining callus as a basis for indirect organogenesis and somatic embryogenesis (Santos, 2005).

For Cuba, the species *S. babylonica* has wide possibilities of ornamental use, in phytoremediation, obtaining secondary metabolites and as an energy biomass. However, it is limited by the scarce presence of this tree, so it is of interest to have in vitro propagation methods. Therefore, the aim of this work was to establish a way for the micropropagation of *S. babylonica* in the different stages of the process under in vitro conditions.

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MATERIALS AND METHODS

Vegetal material

Woody branches of 10 cm long cuttings of the species *S. babylonica* planted in the gardens of the University of Granma were sectioned. The cuttings were placed in glass jars with a volume of 50 ml of distilled water for a period of 15 to 30 days until sprouting, from which the nodal segments were taken (Figure 1).

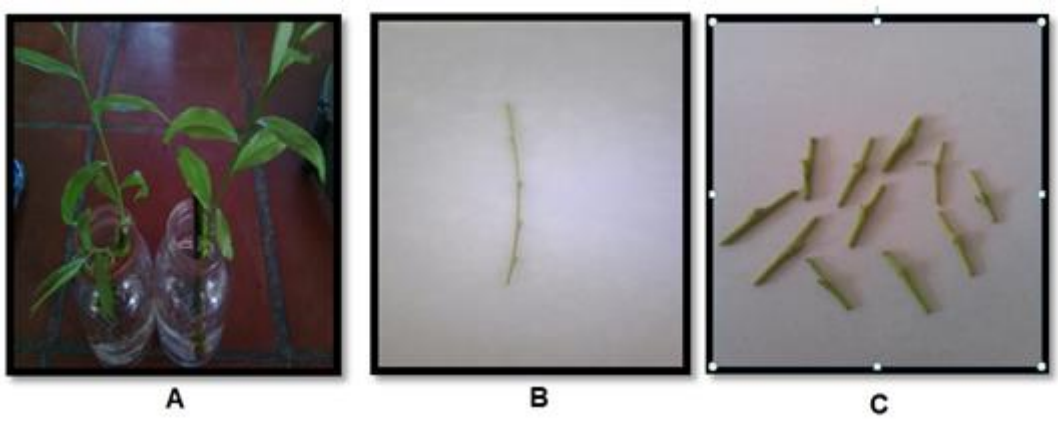

Figure 1. - Obtaining nodal segments of *Salix babylonica*. A) axillary shoots with leaves, B) shoots without leaves to cut the nodal segments, C) nodal segments before disinfection

Culture media and conditions

The basal culture means used to carry out the different experiments was composed of the salts and vitamins MS (Murashige and Skoog, 2006), sucrose 30 g L^{-1}, as gelling agent Agar 6.0 g L^{-1} and the pH was adjusted to 5.7 with pH meter (Crison Basic 20) and the variations were regulated with sodium hydroxide and hydrochloric acid. The culture mean was then distributed in test containers of 24 x 140 mm, at a rate of 10 ml per test container. In experiment III the culture means used are specified.

Sterilization was carried out in a vertical autoclave (BK-75) at 121 ºC temperature and 1.2 kgf.cm^{-2} pressure for 20 minutes. The culture means were kept in the dark for three days before use, in order to detect any type of contamination.

The vegetal material was cultivated on containers using the horizontal laminar flow cabin (FASTER) under aseptic conditions. The growing conditions in the growth chambers with sunlight were: temperature, 25 °C; relative humidity, 70-80 % and in daylight conditions.

Experiment I. Disinfection of the nodal segments of *Salix babylonica* using sodium hypochlorite and mercury bichloride.

The aim of the experiment was to disinfect the *S. babylonica* nodal segments using the different treatments of sodium hypochlorite (NaClO) and mercury bichloride ($HgCl_2$).

The nodal segments were placed in a glass bottle (250 ml) containing a 1 % solution of water and detergent. They were then placed in the magnetic stirrer for 30 minutes to remove sediment and large particles. At the end, they were washed with running water and distilled water. Subsequently, 50 explants were placed in three different bottles for each of the disinfection treatments.

In the laminar flow cabin, the nodal segments were immersed using the three disinfection methods described below:

T1: 1 % active chlorine NaClO for 20 minutes.

T2: 0.1% $HgCl_2$ for 10 minutes.

T3: double disinfection was carried out with 1 % NaClO and 0.1 % $HgCl_2$ for 20 and 10 minutes respectively.

For each of the treatments, four rinses were performed with sterile distilled water, after the application of the disinfectants. They were cut to a length of approximately 1 cm and sown vertically in the culture mean described in that section with the addition of 6-BAP (2.0 mg L^{-1}) at a rate of one explant per test container with 50 explants per treatment.

The evaluation of the results was carried out 14 days after sowing by means of the variable percentages of disinfected explants, contaminated by bacteria, fungi and the necrotic ones.

Experiment II. *In vitro* establishment of *Salix babylonica* nodal segments with the use of different growth regulators

The objective of this experiment was to achieve *in vitro* establishment of *S. babylonica* nodal segments using different combinations of 6-benzylamine purine (6-BAP) with gibberellic acid (AG_3), indolacetic acid (AIA) and naphthaleneacetic acid (ANA) in culture means.

A completely randomized design was used based on a better disinfection result from the previous experiment. Four treatments were used:

T_1: 6-BAP 2 mg L^{-1}

T_2: 6-BAP (2 mg L^{-1}) + AG_3 (5 mg L^{-1})

T_3: 6-BAP (2 mg L^{-1}) + AIA (1 mg L^{-1}) y

T_4: 6-BAP (2 mg. L^{-1}) + ANA (1 mg L^{-1})

Results were evaluated 28 days after planting and the following variables were determined: Percentage of establishment and length of shoot (mm).

Experiment III. Multiplication of *Salix babylonica* nodal segments in three culture media.

The aim of this experiment was to multiply the nodal segments of S. babylonica in three specific culture media.

Nodal segments obtained *in vitro* from the previous experiment were used. The evaluation of the results was made after 28 days of sowing for the following variables: sprouted explants (%) and number of nodal segments per explant.

Table 1. - Culture media used for the multiplication of Salix babylonica nodal segments

Components	Media of Chung and Carrasco (1998)	Media Palomo-Ríos (2015)	MMULT-1
Salts MS	50 %	50 %	100 %
Vitamins MS	100 %	100 %	100 %
Myoinositol	100 mg L^{-1}	100 mg L^{-1}	100 mg L^{-1}
6-BAP	0.1 mg L^{-1}	--	2 mg L^{-1}
AIA	-	-	1 mg L^{-1}
IBA	-	0,1 mg L^{-1}	-
AG_3	1 mg L^{-1}	-	-
Sucrose	30 g L^{-1}	30 g L^{-1}	30 g L^{-1}
Agar	6 g L^{-1}	6 g L^{-1}	6 g L^{-1}
pH	5.7	5.7	5.7

MMULT-1: Means of multiplication

Experiment IV. Induction of *in vitro* rooting of *Salix babylonica* nodal segments with the use of indol-butyric acid (AIB)

The aim of this experiment was to evaluate the effect of different concentrations of AIB on the rooting of nodal segments *of S. babylonica* from the *in vitro* multiplication phase.

For this experiment, nodal segments obtained in the previous experiment in the Palomo-Ríos environment were used. Each treatment consisted of 25 explants. The treatments consisted of the addition to the MS basal culture medium by different concentrations of AIB (0; 0.05 and 0.25 mg L^{-1}). The results were recorded at 28 days of cultivation in the media and the variables assessed were: rooting percentage, number of roots and leaves.

Statistical analysis

A completely randomized design was applied in each of the experiments conducted. For quantitative variables such as number of leaves, number of roots and number of buds, a non-parametric analysis of Kruskal-Wallis was performed, after the data did not respond to the precepts of the analysis of variance. For these analyses, the statistical package InfoStat (Di Rienzo *et al.,* 2014).

For data expressed in percentage, such as explants disinfected, sprouted, contaminated by fungi, bacteria and burned by the disinfectant, sprouting and rooting, we applied the analysis of comparison of proportions with the statistical package CompraPro, according to Castillo and Miranda (2014).

RESULTS AND DISCUSSION

Experiment I. Disinfection of the nodal segments of *Salix babylonica* using sodium hypochlorite and mercury bichloride

As shown in Figure 2, the double disinfection with 1% sodium hypochlorite and 0.1% mercury bichloride for 20 and 10 minutes respectively, was the treatment with the best results for the disinfection of the nodal segments of *S. babylonica*, obtaining 71.4 % of explants disinfected after 14 days, with statistical differences in relation to the other two treatments.

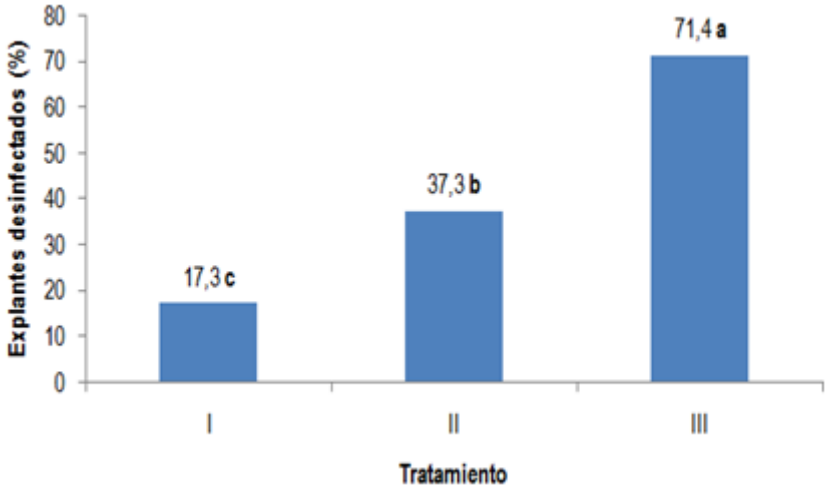

Figure 2. - Effect of sodium hypochlorite and mercury bichloride on the percentage of disinfected explants in *Salix babylonica*. Treatments I-T_1, II-T_2 and III-T_3

It is probably that the concentration and application time of the disinfectants had a favourable influence on the removal of microbial contaminants in the nodal segments of *S. babylonica*, which allowed the spectrum of antimicrobial activity to be extended by double disinfection.

The results of disinfection in this species are varied, depending on the disinfectant agents used. Chung and Carrasco (1998) verified that a mixture of fungicides (Benlate ®-Captan®) and sodium hypochlorite at 10 % for 20 and 30 minutes allowed maintaining aseptic levels higher than 70 % in *Salix sp*. On the other hand, López *et al*., (2016) obtained superior results in the *in vitro* propagation of this same species, obtaining 90% of germ-free explants with the use of sodium hypochlorite at a concentration of 2 % for 15 minutes.

Chornobrov *et al.,* (2019) evaluated in the disinfection of *Salix spp*. as disinfectant agents, mercury bichloride, silver nitrate and sodium hypochlorite in different concentrations and exposure times. The best results were obtained with 85 % of

explants disinfected in a combined treatment of 0.1% $HgCl_2$, then 1 % silver nitrate and 2.5 % sodium hypochlorite for 5-6 minutes in each solution in the order described.

The microbial contamination caused by fungi and bacteria was observed from the seven days of culture, the highest contamination was caused by fungi as shown in table 2. For this microbial group the highest contamination was observed in the treatment with sodium hypochlorite with 63.5 % and with mercury bichloride with 49.0 %.

Table 2. - Effect of sodium hypochlorite and mercury bichloride on the percentage of explants contaminated by bacteria, fungi and necroses in the disinfection of *Salix babylonica* nodal segments

Treatments	Disinfectants	Explants contaminated by bacteria (%)	Explants contaminated by fungi (%)	Necrosed (%)
T1	NaClO (1%)	11,5±0,13	63,5±0,08^b	7,7±0,13
T_2	$HgCl_2$ (0,1%)	9,8±0,10	49,0±0,10^b	3,9±0,13
T_3	NaClO+ $HgCl_2$	10,2 ±0,09	16,3 ±0,13^a	2,0±0,14

Different letters per column differ significantly for $P < 0.05$

For contamination caused by bacteria, no significant differences were obtained between the three treatments evaluated; the range of contamination was between 9.8 and 11.5 %. As an important element, it should be noted that there were no major effects on the explants by necrosis. The values obtained were low, less than 8 %, with no significant differences between the treatments. These results show the importance of the combined use of disinfectant agents that manage to eliminate the two main microbial contaminants of tissue culture, bacteria and fungi, particularly in woody species. For the following experiments, the combined treatment for the elimination of the contaminating microorganisms was used as a method of disinfection.

Experiment II. *In vitro* establishment of *Salix babylonica* nodal segments with the use of different growth regulators

The results of *in vitro* establishment of *S. babylonica* nodal segments are presented in Table 3. Establishment rates ranged from 28.3 to 32.6 % response. The treatments with growth regulators only with BAP, BAP+AG3 and BAP+ANA had significant differences for 5 % with the culture medium with BAP+AIA, according to the ratio comparison analysis. These results demonstrate the complexity of woody plants for *in vitro* cultivation. However, the achievement of these percentages of *in vitro* establishment can be considered a satisfactory result.

For the variable shoot length, there was no significant difference between the treatments, since the values were in the range of 7.54 and 4.42 mm, which shows a small size of the shoots at 28 days of cultivation. These results could be related to

the stress suffered by explants when they are transferred from natural ex-vitro conditions to *in-vitro* conditions.

Table 3. - Effect of different growth regulators on the variables evaluated in the *in vitro* establishment at 28 days of culture

Treatment	Growth Regulators	Establishment (%)	Length of shoot (mm)
T_1	6-BAP (2 mg L^{-1})	31,9±0,12[a]	6,47±3,02
T_2	6-BAP (2 mg L^{-1} +AG_3 (5 mg L^{-1})	31,9±0,12[a]	6,07±1,71
T_3	6-BAP (2 mg L^{-1})+AIA (1 mg L^{-1})	28,3±0,12[b]	7,54±4,14
T_4	6-BAP (2 mg L^{-1})+ANA (1 mg L^{-1})	32,6±0,12[a]	4,40±1,80

Different letters per column differ significantly for $P < 0.05$

It is also considered that there were no significant differences in the results, because in all the culture media 6-BAP was used as cytokinin at a rate of 2 mg L^{-1} and another element that could be related to the response; the use of AIA and ANA at a concentration of 1 mg L^{-1} causes the formation of a basal callus, which affects the development of the axillary bud that originates the nodal segment. It is therefore suggested that future research should use different concentrations of other cytokines such as kinetin, TDZ and the new cytokines of the topoline type, which are difficult to degrade by the action of cytokine oxidases.

Chung and Carrasco (1998), in *in vitro* culture studies of several *Salix species*, used the exogenous application of three concentrations of GA3 (0.1; 0.5 and 1.0 mg L^{-1}) in the presence or absence of 6-BAP; they determined that the best results are obtained with the highest concentration of GA3 (1.0 mg L^{-1}) in the presence of 0.1 mg L^{-1} of 6-BAP, allowing a fast and vigorous development in 76 % of the different origins of the analysed species.

In the species *S. viminalis*, Grendysz *et al*., (2017) successfully established explants of three varieties of the above-mentioned species in the medium Murashige-Skoog (2006), without growth regulators for two weeks until root formation. The nodal segments were then transferred to culture media with kinetin in concentrations of 0.5; 1 and 2 mg L^{-1}. The authors do not present quantitative results, but images that corroborate these good results.

Experiment III. Multiplication of *Salix babylonica* nodal segments in three culture media

Table 4 shows the results of the comparison of the three media. The best results were obtained with the Palomo-Rios culture medium with 0.1 mg L^{-1} of AIB for the variables evaluated with significant differences for 5 % in relation to the other two culture media used, where the percentage of sprouting was 100% and the number of nodal segments was 6.42 at 28 days of culture. On the other hand, the medium of Chung and Carrasco, presenting 6-BAP at a concentration of 0.1 mg L^{-1} and AG_3 at 1 mg L^{-1} had a favourable morphological response of the explants obtaining 88.09 % of sprouting and an average of 1.91 nodal segments. The values reached in the

Palomo and Rios environment for the nodal segments is a favourable indicator for the in vitro propagation of this species. It is expected that each nodal segment will emit new segments when subcultured in the new in vitro multiplication cycles, which should be evaluated in further research.

Table 4. - Influence of different culture media on the sprouting rate and the number of nodal segments in the *in vitro* multiplication of *Salix babylonica L* at 28 days of culture

Treatments	Culture media	Sprouting (%)	Number of SN
T_1	Chung and Carrasco (6-BAP-0,1 mg L^{-1} y AG_3-1 mg L^{-1})	88.09 ±0.05[b]	1.91±1.57[b]
T_2	Palomo-Rios (AIB-0,1 mg L^{-1})	100± 0.00[a]	6.42±2.32[a]
T_3	MMULT-1 (6-BAP-2 mg L^{-1} and AIA-1 mg L^{-1})	54.71 ±0.10[c]	0.00±0.00[c]

Different letters per column differ significantly for $P < 0.05$

The Palomo-Ríos and Chung and Carrasco propagation media only present 50 % of the Murashige-Skoog salts, which favours that the nodal segments had a better *in vitro* response with this salt concentration and with low concentrations of the growth regulators used, unlike the MMULT-1 media where the Murashige Skoog salts were used at 100% and the BAP and AIA concentrations were higher.

Palomo *et al.,* (2015) described a method of micropropagation that could generate seedlings on the order of 5000 viable and transplantable clones from a single plant in just 24 weeks and was used to produce export-tested plant breeding material to overcome the restriction on international transport of woody cuttings. This method could represent a valuable biotechnological complement to willow breeding programs and could accommodate early selection by molecular or biochemical markers.

Figure 3 shows the development of the explants in each of the treatments used, which shows that not all had the same response in vitro, noting that the seedling in the middle Palomo-Ríos (T_2) the shoot has a greater growth with emission of roots and leaves with a greater foliar area. In the Chung and Carrasco medium (T_1) the shoot, roots and leaves show less development.

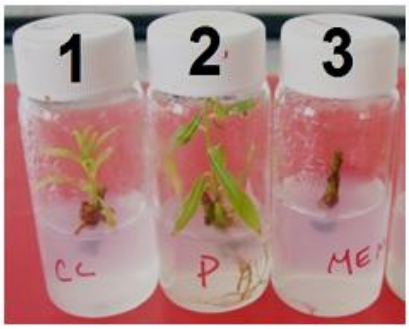

Figure 3. - *In vitro* plants of *Salix babylonica* in the different culture media used in multiplication, 1-1-T_1, 2-T_2 and 3-T_3

According to the results obtained in this same species by López *et al.,* (2016), the low concentrations of growth regulators used as 6-BAP (0.045 mg L^{-1}), ANA (0.020 mg L^{-1}) and AG_3 (0.050 mg L^{-1}) were efficient, since they promoted a greater number of shoots and length per explant with 5.6 and 12.4 mm, respectively.

Other authors, such as Chung and Carrasco (1998), used different doses of AG_3 (0.1; 0.5 and 1.0 mg L^{-1}) in the presence or absence of BAP (0.1 mg L^{-1}) and BAP alone in the micropropagation of leaf meristems of different species of Salix spp. in the multiplication and rooting phase. They determined that the treatments that gave the best results were: the use of 1.0 mg L^{-1} of AG_3 and 0.1 mg L^{-1} of BAP and the combination of both hormonal concentrations.

In research carried out by Regueira *et al*., (2018) on the species *S. viminalis*, they determined that the type of explant influenced the quality of the shoot and the multiplication coefficient, with basal segments showing a greater capacity for proliferation than the apical and middle shoot segments. Willow shoots grown in containers plantform™ and RITA® produced higher multiplication coefficients than shoots grown in semi-solid medium.

In summary, the Palomo-Ríos medium achieved 100 % sprouting and an average number of nodal segments with a significant difference in relation to the other two media used.

Experimento IV. Rooting induction in nodal segments of *Salix babylonica* using indol-butyric acid (AIB)

The evaluation of the rooting of the shoots obtained from the multiplication showed that in the analyzed variable percentage of rooting, there was a significant difference between all the evaluated treatments, being the treatment 1 without growth regulators where the highest percentage was obtained, with 91.66. These results may be related to the presence of endogenous auxins that favour rooting, since it was observed that when semi-yellowish cuttings were placed in distilled water to obtain the shoots for in vitro cultivation, the cuttings were able to form roots.

In the evaluation of the number of leaves and roots, the best results were obtained in T_1 and T_2 with an average of 4.27 and 5.04 for the first variable and for the second the values were 1.88 and 2.67, with significant differences for 5 % with T_3 for both variables, as can be seen in Table 5.

Table 5. - Effect of different AIB concentrations on rooting percentage, number of leaves and roots in the rooting phase at 28 days of cultivation

Treatment	AIB (mg L^{-1})	Percentage of rooting	Number oy leaves	Number of roots
T_1	0,00	91,66±0,04[a]	4,27±1,00[a]	1,82±1,71[a]
T_2	0,05	78,26±0,06[b]	5,04 ±1,77[a]	2,67±1,90[a]
T_3	0,25	9,52±0,14[c]	1,95±1,60[b]	0,43±1,16[b]

Different letters per column differ significantly for $P < 0.05$

These results show that the increase in AIB concentration reduced the favourable behaviour in root induction, which caused a tendency to inhibit the rooting process, as shown in Figure 4.

Figure 4. - *In vitro Salix babylonica* plants rooted in MS culture medium at 28 days of culture

Other authors, such as López *et al*., (2016), obtained the best results at this stage in this same species, using 1 mg L^{-1} of AIA, since it promoted the greatest number and length of roots with 2.19 and 2.41 respectively.

On the other hand, Naujoks (2007) induced root formation in *S. caprea* with an AIB treatment at 50 mg L^{-1} for three weeks and then transfer to a culture medium without auxins for root development.

Gómez *et al.*, (2014) obtained excellent results when they used AIB at 0.5 mg L^{-1} in the in vitro rooting of the plant *Populus tremula*, a species of the same family as *S. babylonica*.

The results indicate that the use of AIB-type auxins is not necessary for the induction of roots obtained in vitro from the species studied.

CONCLUSIONS

The results achieved in the investigation allow us to have a protocol for the *in vitro* propagation of *Salix babylonica* that covers the stages of disinfection of explants, establishment, multiplication and in vitro rooting. The disinfection of nodal segments was obtained with a double disinfection, first with 1 % sodium hypochlorite and then 0.1 % mercury bichloride for 20 and 10 minutes, respectively, with 71 % of nodal segments disinfected. In vitro establishment was achieved in the culture medium only by using BAP 2 mg L^{-1} as 31.9 % of establishment was obtained. Nodal segment multiplication was higher in Palomo-Ríos culture medium with 100 % sprouting and 6.42 nodal segments. Rooting was obtained in basal culture medium without AIB with 91.6 % response.

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The authors declare not to have any interest conflicts.

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The authors have participated in the writing of the work and analysis of the documents.

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