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Response of Red Maple (*Acer rubrum* L.) Micropropagation to Different *In Vitro* Conditions

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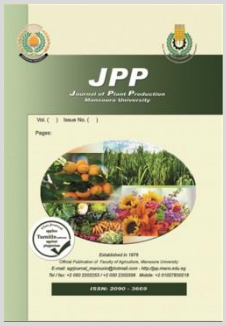

ABSTRACT

A reliable micropropagation protocol was developed for Red Maple tree for the first time in Iraqi Kurdistan Region to introduce this newly entered species to the local community. At initiation stage, healthy and vigorous cultures were established following a critical disinfestation protocol by bleach (Sodium hypochlorite) at 2.5 % for 20 minutes. The obtained results show that shoots multiplication was successfully enhanced by recording the highest number of shoots and leaves per explant when 0.5 mg.L^{-1} BA was added to WPM by recording 2.08 shoots/ explant and 13.7 leaves/ explant, respectively. Whereas, the longest shoots were obtained when 1.5 mg.L^{-1} BA was added by reaching 1.86 cm. For root formation, IBA was more effective than NAA by giving the best rooting parameters. The highest number of roots per explant (3.00 roots/ explant), the longest roots (5.87 cm) and the highest rooting percentage (100%) were obtained when 0.1 mg.L^{-1} IBA was added to WPM. The half-light intensity (50 feet/ candle) showed better rooting abilities of microshoots than full light intensity conditions (100 feet/ candle) by raising the number of roots from 3.00 to 4.00 roots/ explants, increasing the mean length of roots from 5.87 cm to 6.37 cm when the comparing between the highest rooting parameters at full light illumination. A 100% survival rate was achieved from the acclimatized plantlets in the greenhouse conditions without any abnormal morphological characteristics. It can be recommended that this important plant can be propagated by tissue culture technique toward mass production in the local area.

Keywords: Red Maple, *Acer rubrum* L., Micropropagation, *In Vitro* conditions

INTRODUCTION

Maple is a famous category of trees and shrubs that are distributed in the northern hemisphere ranging from America to Japan including Europe, Middle East, North Africa and Central Asia (Gibbs and Chen, 2009). Red maple and swamp maple are the common names of *Acer rubrum* L. which belongs to Aceraceae family. It is called red maple because of its red flowers, fruits and leaves especially in fall season. It is recommended to plant the red maples as buffer strips in parking lots and median strips in Highway streets as well as in parks and gardens that's why it is called garden or street tree (Gilman and Watson, 1993). Red maple is a fast-growing tree comparing with other Maple species. However, it can grow in both shade and sunny places as well as in different kinds of soil as clay, sand, loam and well drained soils and in both dry and wet soils, but it prefers wet ones (Stevens, 1999). In addition of using the red maple as a landscape tree, it is also used in lumber manufacture, high quality paper manufacture, fire wood, maple syrup production and provide shelter and food for some animals as birds and squirrels (Gilman and Watson, 1993; Wann and Gates, 1993 and Dickerson, 2002). Red maple is commercially propagated by seeds, cuttings and budding but these methods face many problems for instance the survival seedlings produced seeds is less than 50% and the survivors usually have bad characters as poor growth and bad root system (Abbott and Verrier, 1965). Furthermore, due to the bud union incompatibility, loses may reach to 70% through the first and the second year (Abbott and Verrier, 1965; Schwab, 1979 and Moller, 1985). Moreover, generally maple stem cuttings are hard to root, and the plants produced are in bad quality (McClelland *et al*., 1990). To overcome these problems, propagation via the techniques of tissue culture is the best solution. plant tissue culture overwhelmingly used to preserve the genotype in demand as well as to produce individuals that have the same phenotype. For ornamental purpose, red and silver maple have been *in vitro* propagated while for wood production, other types of maple have been *in vitro* propagated as pig tooth maple (Ďurkovič and Mišalova, 2008). Several researchers used shoot tips, buds and nodal segments as explants for maple micropropagation (Preece *et al*. 1991; Wann and Gates 1993 and Ďurkovič 1996). Wann and Gates (1993) used the combination between BA and TDZ for regeneration of red maple. However, Orlikowska and Gabryszewska (1995) found that TDZ at low concentration was better for multiplication of red maple and shoot necrosis was appeared when BA was used. Furthermore, IBA is the best auxin for *in vitro* rooting of red maple (Orlikowska and Gabryszewska, 1995 and Zhou, 2018). Moreover, McClelland *et al*. (1990) compared between *in vitro* and *ex vitro* root cuttings, and they found that the *in vitro* roots had better quality and were healthier than *ex vitro* ones as well as the survival rates were higher. Nevertheless, using Kinetin in low concentration gave best *in vitro* rooting results (Hanus and Rohr, 1987). The main objectives of the study were to find out a reliable micropropagation protocol

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for this forest and ornamental tree by testing different plant growth regulators and to introduce it to the local community in Iraqi Kurdistan Region.

MATERIALS AND METHODS

Red maple cuttings were received from UK in winter season. The experiments were conducted at Plant Tissue Culture labs belong to College of Agricultural Engineering Sciences in University of Duhok, Iraqi Kurdistan Region. The cuttings were forced using $Mg.L^{-1}$ BA and $Mg.L^{-1}$ GA_3 for ten days in refrigerator. After buds flushing up, explants were taken and washed by tap water and dish washing liquid solution for 40 minutes. The disinfestation then was completed under aseptic conditions in the Laminar-Air-Flow cabinet. Explants were sterilized first by 70% ethanol alcohol for 1 minute then rinsed three times with sterilized distilled water each for three minutes followed by sterilization by bleach (Sodium hypochlorite) at 2.5 % for 20 minutes. Finally, they were rinsed with sterilized distilled water three times and each for three minutes. After completing disinfestation, the damaged ends were cut out and the explants were inoculated on WPM supplemented with 1 $Mg.L^{-1}$ of both BA and GA_3. The cultures were kept in growth room under 25± 2°C and illuminated with 16 hours daily with white cold light at 100 feet/ candle light intensity. After 6 weeks on initiation stage, the mictoshoots were subcultured on multiplication medium enriched with BA and Kin at 0, 0.5, 1.0, 1.5 and 2 $Mg.L^{-1}$. At the end of shoot multiplication stage, number of shoots, mean length of shoots and number of leaves per explant were recorded after 6 weeks in culture. For roots formation stage, different concentrations of IBA and NAA were tested at 0, 0.1, 0.5, 1.0 and 1.5 $Mg.L^{-1}$ as well as two light intensities (50 and 100 feet/ candle) were tested. At the end of rooting stage, rooting percentages, number of roots per explant and mean length of roots were recorded after 6 weeks in culture. Finally, the produced plantlets were gradually acclimatized by planting in small plastic pots containing peatmoss and perlite at 1/0.5 V/V and then moved to the greenhouse conditions. The experiments were designed according to Completely Randomized Design (CRD) in nine replicates and the comparisons among means were done according to Duncan multiple range test at 0.05 (SAS, 2010).

RESULTS AND DISCUSSION

The forcing treatment with 10 MgL^{-1} GA_3 and BA was very effective in promoting the dormant buds to flush up in winter time. Healthy cultures were successfully established by the effective disinfestation treatments done by immersing the explants in 70% ethanol alcohol for one minutes followed by immersing in 2.5% of sodium hypochlorite for 20 minutes.

Table 1 illustrates the effect of BA and Kinetin on multiplication stage of red maple after six weeks in culture on WPM. In general, BA was better than Kinetin in enhancing explants proliferation by giving the best parameters. The highest number of shoots and leaves per explant were achieved when 0.5 $mg.L^{-1}$ BA was added by recording 2.08 shoots/ explant and 13.7 leaves/ explant respectively. However, the longest shoots were obtained when 1.5 $mg.L^{-1}$ BA was added by reaching 1.86 cm. On the other, the least number of shoots per explant, the shortest shoots and least number of leaves per explant were achieved when 2 $mg.L^{-1}$ Kinetin was added which produced 0.33 shoots/ explant, 0.50 cm and 2 leaves/ explant respectively (Figures 1, 2 and 3).

Table 1. The response of Red Maple (*Acer rubrum* L.) shoot multiplication to different concentrations of Benzyl Adenine and Kinetin after six weeks in culture on WPM medium

Treatments	Concentrations ($Mg.L^{-1}$)	Number of shoots /explant	Mean length of shoots (cm)	Number of leaves/explant
Control	0	0.66 cd	0.50 c	2.44 d
BA	0.5	2.08 a	1.73 a	13.7 a
	1.0	1.33 b	0.94 b	6.92 c
	1.5	1.58 b	1.86 a	10.5 b
	2.0	1.00 c	1.16 ab	5.70 c
Kin	0.5	1.00 c	1.54 a	4.66 c
	1.0	0.91 c	1.29 ab	4.83 c
	1.5	0.80 c	0.80 bc	3.00 d
	2.0	0.33 d	0.50 c	2.00 d

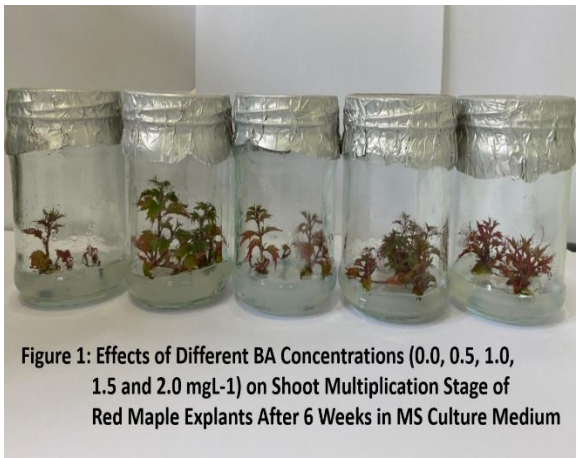

Figure 1: Effects of Different BA Concentrations (0.0, 0.5, 1.0, 1.5 and 2.0 mgL-1) on Shoot Multiplication Stage of Red Maple Explants After 6 Weeks in MS Culture Medium

Figure 2: The best shoot multiplication treatment (BA 0.5 mg.L-1) of Red Maple Explants After 6 Weeks in MS Culture Medium

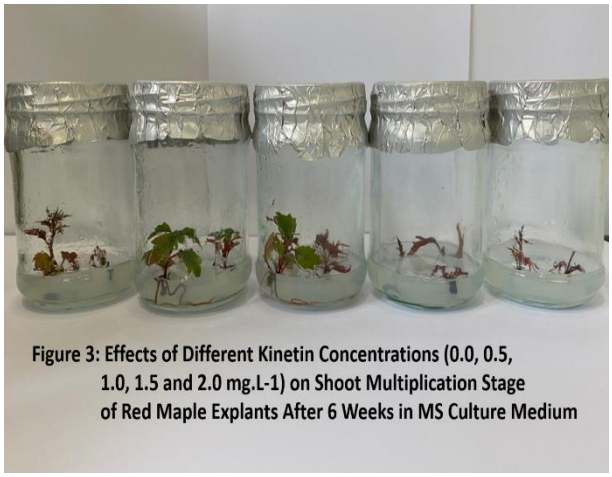

Figure 3: Effects of Different Kinetin Concentrations (0.0, 0.5, 1.0, 1.5 and 2.0 mg.L-1) on Shoot Multiplication Stage of Red Maple Explants After 6 Weeks in MS Culture Medium

The cytokinins enhanced role in shoot multiplication of the plant is largely due to their effects on the release of apical and lateral buds from the apical dominance of the terminal buds without the need for apical bud cutting by promoting the formation of xylem and phloem in the buds. That facilitates the conversion of nutrients and water, leading to the growth of unfinished buds (Mohammed and Al-Younis, 1991). Besides their significant role in the biosynthesis of RNA, enzymes and proteins in the plant cell that also promotes bud growth (Al-Rifae'e and Al-Shobaki, 2002). Moreover, the structure of hereditary plant organs has an influence on the response of the cultured plant due to its effect on the content of endogenous hormones (Singh *et al*., 1994). The general conclusion drawn from the plant multiplication results is that Kinetin was less effective than BA at the same concentrations added to the multiplication culture media. The reasons for the superiority of BA may be due to its molecular structure and the number of double bonds on its side chain from the benzyl ring (Mohammed, 1985). In addition, BA is considered to be the most effective cytokinin in cell division and overcoming apical dominance in comparison to other cytokinins (Bashi, 2006; Fadladeen and Toma, 2020).

It was noticed that the addition of BA to the shoot multiplication medium caused abnormal morphological effects on leaves. This abnormality increased by in raising the concentration of BA added (Figure 4). This might due to the strong effect of BA as compared to Kinetin and the sufficiency of endogenous cytokinins level in explant tissues.

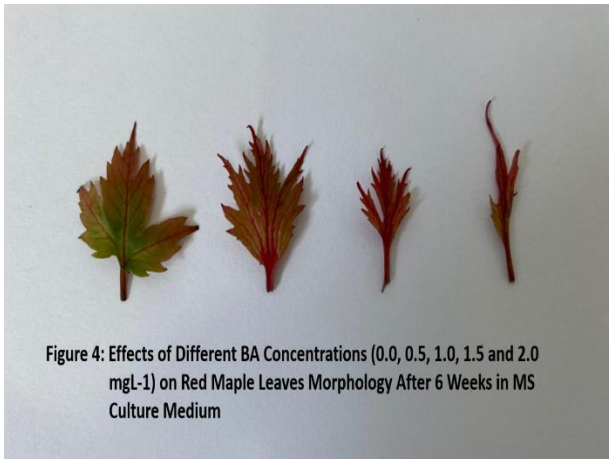

Figure 4: Effects of Different BA Concentrations (0.0, 0.5, 1.0, 1.5 and 2.0 mgL-1) on Red Maple Leaves Morphology After 6 Weeks in MS Culture Medium

Table 2 shows the response of Red Maple (*Acer rubrum* L.) roots formation to different concentrations of NAA and IBA after six weeks in culture on WPM grown under full light intensity. Generally, IBA was more effective than NAA by giving the best parameters. The highest number of roots per explant, the longest roots and the highest rooting percentage were obtained when 0.1 mg.L^{-1} IBA was used which gave 3.00 roots/ explant, 5.87 cm and 100% rooting respectively (Figure 5).

Table 2. The response of Red Maple (*Acer rubrum* L.) root formation to different concentrations of NAA and IBA after six weeks in culture on WPM medium grown under full light intensity.

Treatments	Auxins (Mg.L^{-1})	Number of roots/explant	Mean length of roots (cm)	Rooting percentage
Control	0	1.11 c	1.75 d	50%
NAA	0.1	1.25 c	1.66 d	50%
	0.5	0.83 d	1.75 d	50%
	1.0	2.30 b	1.66 d	75%
	1.5	2.2 b	4.7 b	70%
IBA	0.1	3.00 a	5.87 a	100%
	0.5	2.50 b	3.56 c	75%
	1.0	2.25 b	4.62 b	75%
	1.5	0 d	0 e	0

Figure 5: Root formation on Red Maple microshoots grown on WPM supplemented with 0.1, 0.5, 1.0 and 1.5 mgl-1 IBA after 6 weeks in culture.

Table 3 shows the effect of NAA and IBA on rooting stage of red maple after six weeks in culture on WPM medium grown under half-light intensity. Generally, NAA was better than IBA with half-light intensity. The best parameters were recorded when 0.5 mg.L^{-1} NAA was added by giving 4.00 roots per explant, 5.37 cm and 100% rooting percentage. On the other hand, there was no roots induction with the same auxin at 1.5 mg.L^{-1} (Figure 6).

Table 3. The response of Red Maple (*Acer rubrum* L.) root formation to different concentrations of NAA and IBA after six weeks in culture on WPM medium grown under half- light intensity.

Treatments	Auxins (Mg.L^{-1})	Number of roots/explant	Mean length of roots (cm)	Rooting percentage
Control	0	0.83 d	1 c	50%
NAA	0.1	1.50 c	6.37 a	75%
	0.5	4.00 a	5.37 b	100%
	1.0	1.50 c	4.37 b	100%
	1.5	0 e	0 e	0
IBA	0.1	2.25 b	3.99 b	75%
	0.5	1.75 c	3.00 b	75%
	1.0	0.75 d	1.16 c	25%
	1.5	0.25 d	0.25 d	25%

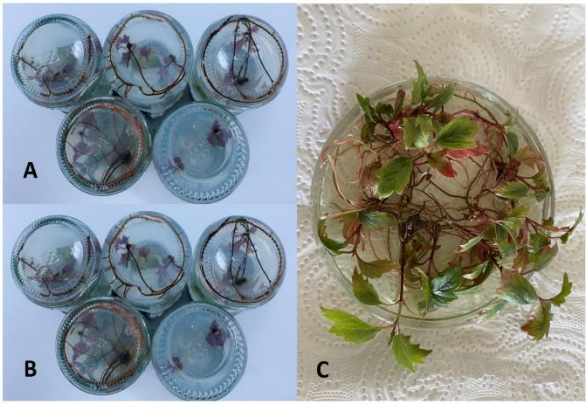

Figure 6: Effect of (A and C) full and (B) half light intensity on root formation on Red Maple microshoots grown on WPM supplemented with 0.0, 0.1, 0.5, 1.0 and 1.5 mgL-1 IBA after 6 weeks in culture.

In general, the positive effects of auxins in enhancing root formation is due to the major physiological effects of the auxins is the stimulation of adventitious roots formation in both *in vitro* and *in vivo* cuttings (Hartmann *et al*., 2002). In conclusion, rooting results obtained in this investigation confirmed the need for auxins for red maple adventitious root formation. These results indicated that the presence of auxins had positive influences on rhizogenesis in micro-shoots rooting *in vitro*. In addition, the most effective auxin in the rooting of red maple was IBA at full light intensity and NAA at half-light intensity. Such differences in the potency of auxin in inducing rooting might attributed to the structure of the auxins under study, the endogenous hormone level, as well as the genetic makeup of species under consideration. This also explains the different response of different light intensities based on these variations (Karhu and Zimmerman, 1993).

A hundred percent successful acclimatization process was achieved for the plantlets hardened-off and gradually moved to greenhouse conditions (Figure 7). No abnormal morphological and physiological growth were found on the red maple acclimatized plantlets.

Figure 7: Acclimatization stage of Red Maple plantlets grown in small plastic pots containing peatmoss and perlite at 1/0.5 V/V after two weeks in the greenhouse.

In conclusions, this study confirmed the ability of a successful micropropagation of this important forestry and ornamental tree by raising the rooting percentage to reach 100% under *in vitro* conditions. Besides, overcoming the obstacles facing the conventional propagation methods like poor rooting and bad quality of seedlings. The half-light intensity showed better rooting ability of microshoots by raising the number of roots per explant from 3.00 to 4.00 roots/ explants, increasing the mean length of roots from 5.87 cm to 6.37 cm when the comparing between the highest rooting parameters at full light illumination. These results confirm that red maple microshoots need lower light intensity which in result will reduce the costs of lighting in mass micropropagation.

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إستجابة الاكثار الدقيق لشجرة القيقب الأحمر (*Acer rubrum* L.) لظروف وعوامل مختلفة للإكثار خارج الجسم الحي

ليلان حسين فضل الدين ، روفائيل شليمون توما ، ميديا أحمد محمد ، أحمد شاهين و هدى برهان أحمد

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تم استحداث برنامج اكثار دقيق لشجرة القيقب الأحمر للمرة الأولى في اقليم كوردستان العراق للعمل على إدخال وتعريف هذه الشجرة الهامة للمجتمع المحلي. في مرحلة النشوء، تم الحصول على مزارع معقمة وقوية من خلال إتباع برنامج تعقيم متكامل للأجزاء النباتية باستخدام ٢,٥% من هيبوكلورات الصوديوم لمدة ٢٠ دقيقة. أشارت النتائج التي تم التوصل اليها الى أنه تم تحفيز مرحلة التضاعف الخضري بنجاح من خلال اضافة ٠,٥ ملجم/ لتر من البنزيل أدنين إلى وسط الزراعة WPM عن طريق الحصول على أكبر عدد من الأفرع لكل جزء نباني (٢,٠٨ فرع/ جزء نباتي) وأكبر عدد من الأوراق (١٣,٧ ورقة/ جزء نباتي) على التوالي. بينما تم الحصول على أطول الفروع عند اضافة ١,٥ ملجم/ لتر من البنزيل أدنين والتي وصلت أطوالها الى ١,٨٦ سم. لتحفيز تكوين الجذور على الأفرع الناتجة، وجد بأن اضافة اندول حامض البيوتريك كان أكثر فعالية من اندول حامض النفثالين من خلال تسجيل أفضل النتائج لمقاييس التجذير. تم تسجيل أكبر عدد من الجذور (٣,٠٠ جذور/ جزء نباني) وأطول الجذور (٥,٨٧ سم) واعلى نسبة تجذير (١٠٠%) عند اضافة ٠,١ ملجم/ لتر من الأندول حامض البيوتريك إلى وسط WPM. من جهة أخرى، فإن حضن النبيتات الناتجة على ظروف نصف شدة الاضاءة (٥٠ شمعة/ قدم)، أظهرت امكانية أفضل للتجذير عند مقارنتها مع تلك النامية في ظروف شدة الاضاءة الكاملة (١٠٠ شمعة/ قدم) من خلال ارتفاع عدد الجذور المتكونة من ٣,٠٠ الى ٤,٠٠ جذور/ جزء نباتي وزيادة معدل أطوالها من ٥,٨٧ سم الى ٦,٣٧ سم. أما في مرحلة التقسية والنقل الى خارج المختبر، فقد تم تسجيل نسبة نجاح عالية جداً وصلت الى ١٠٠% تحت ظروف البيت الزجاجي بدون ظهور أي أعراض غير طبيعية من حيث المظهر الخارجي أو الفسيولوجي. وعليه بالإمكان التوصية بإكثار هذا النبات الهام بتقنية الزراعة النسيجية نحو الانتاج الكمي في المنطقة.

الكلمات دالة: القيقب الأحمر، *Acer rubrum* L.، الإكثار الدقيق، الزراعة النسيجية