In Vitro Propagation of *Pyracantha coccinea* as Affected by Growth Regulators and Different Carbon Sources.

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Abstract

The aim of this study was to investigate the effect of different types and concentration of plant growth regulators (Cytokinins: Kinetin and Benzyl adenine (BA) at 0, 0.5, 1, 1.5, 2 and 2.5 $mg.l^{-1}$) on shoot proliferation of *Pyracantha coccinea* and auxin (indole-3- butyric acid) (IBA at 0, 0.5 and 1 $mg.l^{-1}$) and different carbon sources (Sucrose and Glucose) with ½ and full MS salt strength in root formation. The results obtained from this study indicate that the greatest number shoots and leaves per explant were acquired when BA was 2.5 mg/l^{-1}, which gave 8 shoots and 54.33 leaves respectively. In the rooting stage, the greatest number of roots per explant was obtained when half MS salt strength was used with Sucrose and $1mg.l^{-1}$ IBA, which gave 11.33 roots/explant. Up to 83% of shoots were rooted. plantlets of *P. coccinea* with roots were successfully acclimatized with a survival rate of 80% in sand, 95% in peatmoss and 85% in sand + peatmoss. This procedure can be adopted for *P. coccinea in vitro* propagation.

Key words: *Pyracantha coccinea*, PGRs, carbon sources.

تأثير منظمات النمو النباتية ومصادر الكاربون على إكثار نبات *Pyracantha coccinea* خارج الجسم الحي

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الملخص

كان الهدف من هذه الدراسة هو اختبار تأثير الأنواع المختلفة من منظمات النمو النباتية (السيتوكينين Kinetin :و Benzyl adenine (BA) بالتراكيز 0، 0.5، 1، 1.5، 2 و 2.5 ملغم. لتر $^{-1}$ على اكثار نبات *Pyracantha coccinea* ، وكذلك الأوكسين (حمض الإندول بيوتاريك، IBA) عند 0 ، 0.5 و 1 ملغم. لتر $^{-1}$ ومصادر مختلفة للكربون (السكروز والجلوكوز) مع ½ وكامل قوة املاح MS في تكوين الجذور. تشير النتائج التي تم الحصول عليها من هذه الدراسة إلى أن أكبر عدد من الافرع والأوراق لكل نبات تم الحصول عليه في المعاملة التي استخدم فيها BA 2.5 ملغم. لتر $^{-1}$ والتي اعطت 8 أفرع و54.33 ورقة على التوالي. في مرحلة التجذير، تم الحصول على أكبر عدد من الجذور لكل جزء نباتي عندما تم استخدام الوسط موراشج وسكوك (MS) بنصف قوة املاحه مع السكروز و1 ملغم. لتر $^{-1}$ IBA، والتي أعطت 11.33 جذر / جزء نباتي. وصلت النسبة المئوية إلى 83٪ من البراعم. تم اقلمت نباتات *P. coccinea* المجذرة بنجاح بمعدل نجاة بلغت 80٪ في الرمل و95٪ في البيت موس و85٪ في الرمل + البيت موس. يمكن اعتماد هذا الإجراء في حالة اكثار نبات.*P. coccinea* خارج الجسم الحي.

الكلمات الافتتاحية: *Pyracantha coccinea*، *منظمات النمو النباتية*، مصادر الكاربون.

Introduction:

The garden shrub *Pyracantha coccinea* M. Roem. It is a Rosaceae family member and an evergreen thorny shrub that is a native species to Iraqi Kurdistan. The common names of this species are scarlet firethorn as an English name, Guhyshka bakhja as a Kurdish name, and Zaaror al-Zena as an Arabic name (Shahbaz, 2010). This native species is present at about 750 m above sea level, starting from Bikhair Mountain to the north of the region in open pine and oak forests (Shahbaz, 2010). However, it is also distributed in other regions, such as Turkey, Iran, the Caucasus, Southeast Europe and Southeast Asia (Fico *et al*., 2000). The attractive habit of this

shrub with white flowers, red fruits and a neat shoot system shape makes this species an excellent choice for urban gardens. Flowers blooming in the spring, and later in the fall, the meaty orange to dark red berries ripen, which are primarily consumed by birds. Jellies, jams, sauces, and marmalade can all be made from ripe berries (Quiroga *et al*., 2003). This plant has been used as a stomachic, expectorant, and aperient for centuries. Furthermore, it currently exhibits a variety of pharmacological properties, including antioxidant, cytotoxic, radioprotective, mutagenic and antimutagenic, antibacterial, and enzyme inhibitory properties (Al-Snafi, 2016). *Pyracantha coccinea* is valuable not just as a shade tree, but also for its nutritional and therapeutic characteristics. Scarlet firethorn fruits are used as a diuretic and a heart tonic, and their fruits have therapeutic characteristics (Kowaleuki and Mrugasiewicz,1971). *Pyracantha coccinea* is rarely grown by traditional methods, both sexually as seeds and vegetative as cuttings, due to its seeds twofold dormancy, which necessitates stratification, and cuttings' difficulty in rooting (Dirr and Heuser, 2009). To overcome these problems, plant tissue culture is an excellent protocol to propagate plants (Toma, 2018). Plant tissue culture is an easy and rapid propagation technique used for plants that have problems with traditional methods (Offord and Tyler 2009, Goncalves *et al.* 2010). For the proliferation of *Pyracantha coccinea*. Chao et al (2017) have used (MS) basal medium augmented with different BA concentrations, as well as rooting media with varied concentrations of IBA, using MS medium supplemented with 1.5 $mg.l^{-1}$ BA, improved establishing rate 67%, 3/4 basal MS supplemented with 0.3 $mg.l^{-1}$ IBA was the optimum medium for shoot proliferation whith Proliferation index of 3.4. 1/4 MS basal medium with 1.9 $mg.l^{-1}$ IBA was shown to be the best rooting medium. The percentage of shoots that were rooted was as high as 77 percent. Norton and Boe (1982) employed MS supplemented with BA at various concentrations alone or in combination with other growth regulators for the micropropagation of cotoneaster spp. Monier and Ochatt (1995) found that 1 $mg.l^{-1}$ was the best For the multiplication stage, when investigating the impact of BA on the *in vitro* propagation of five cotoneaster genotypes. In addition, Sivanesan *et al*. (2011) employed MS medium enriched with TDZ at 0.5 $mg.l^{-1}$ and NAA at 0.1 $mg.l^{-1}$, resulting in a 77 percent success rate. This study's principal objective was to develop a viable in vitro reproduction and rooting protocol for *Pyracantha coccinea* by testing several effective factors including growth regulators, carbon sources and salt strength of culture medium.

Materials and Methods:

This study was carried out in the laboratory for plant cell and tissue culture, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan Region of Iraq. The nodes which were used as explants were collected during May to July from one of the private commercial nurseries. They were washed with tap water for 30 minutes and every 10 minutes they were treated with a few detergent drops of washing up liquid. After that, the explants were surface sterilized by immersion in 10% sodium hypochlorite for 10 minutes. Afterward, they were given three sterile distilled water rinses, each lasting ten minutes. Next, they were cut into single nodal segments and 1.0 cm and cultured on initiation medium composed of MS medium (Murashige and Skoog,1962) free of plant growth regulators. BA and Kin were added to the MS medium to enhance the multiplying stage at 0, 0.5, 1, 1.5, 2, and 2.5 $mg.l^{-1}$. The recorded characteristics for this stage included the quantity of

shoots per explant, average shoot length (cm), and quantity of leaves per explant For the rooting phase, 30 g of two kinds of carbon sources (sucrose and glucose) were used with ½ and full MS salt strength and IBA at 0, 0.5 and 1 $mg.l^{-1}$. After adjusting the pH to 5.75 with 1 N NaOH or 1N HCl and adding agar (7 $g.l^{-1}$), the media was autoclaved for 20 minutes at 121ºC and 117.68 kPa. For each treatment, ten shoots were prepared. The microshoots were inculcated at 25º ± 2º C, with a 100 mmolm–2s–1 light intensity and a 16/8 h light/dark light cycle. Newly produced shoots were employed by subsequent researchers after 30 days. The parameters length of roots (cm), number of roots/explant, and rooting rate were taken 4 weeks later. For the acclimatization stage, three kinds of media were used: sand, peat moss and sand with peat moss. Plantlets were transplanted into a greenhouse and irrigated as necessary. Plants that are present survived after four weeks was counted. The statistical analysis for the investigation was as a Complete Randomized Design constructed (CRD). According to the Duncan's Multi-Range Test (at 5%).

Results and Discussion:

Pyracantha coccinea explants were successfully initiated with 95%. The mean shoot parameters: 4 branches, 1.9 cm length and 26 leaves.

The effects of kinetin and BA on the initial phase of *Pyracantha coccinea* explants grown on MS media following six weeks are shown on Table 1. Generally, BA was better than Kinetin for shoot initiation and multiplication. The greatest volume of shoots and leaves per explant was acquired when 2.5 $mg.l^{-l}$ BA was used, which gave 8 shoots and 54.33 leaves per explant respectively. Whereas the fewest shoots per explant were obtained in the control treatment by giving 1 $shoot/explant^{-1}$. However, the lowest number of leaves per explant was obtained when 0.5 mg of l-l kin was applied, 8.33 $leaves/explant^{-1}$ were produced. On the other hand, the longest shoot was acquired when 2 $mg.l^{-l}$ Kin was used, which was 3 cm, while the shortest shoot was obtained via using 2.5 mg/l-l Kin and providing shoots that were 2 cm long.

Among the most critical restrictions to woody plants species reproduction is bud dormancy, that often solved via employing cytokinin. The incorrect concentration of cytokinin, on the other hand, frequently induces the explant becoming vitrified (Hanover and Keathley, 1988).

Table (1): Effect of kinetin and BA on initiation stage of *Pyracantha coccinea* explants cultured on MS medium after six weeks.

Cytokinins	Concentrations	No: shoots/ explant	Mean length of shoots (cm)	No: leaves/ explant
Control	0	1.00 f	2.83 a	9.67 f
Kin	0.5	1.67 e	2.67 a	8.33 f
	1	2.33 d	2.52 a	15.67 e
	1.5	3.33 c	2.83 a	22.00 c
	2	1.67 e	3.00 a	14.33
	2.5	1.67 e	2.00 b	15.00 e
BA	0.5	3.00 c	2.67 a	22.33 c
	1	3.00 c	2.53 a	19.00 d
	1.5	4.67 b	2.60 a	34.00 b
	2	4.33 b	2.30 ab	32.33 b
	2.5	8.00 a	2.20 ab	54.33 a

*Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

Table (2) shows the effect of carbon source, MS salt strength and IBA concentrations on the rooting stage of cotoneaster explants after six weeks in culture. According to the data in the table three of the treatments gave the best rooting rate, which was 100%. Those treatments were full and half MS salt strength with the use of sucrose and 0.5 $mg.l^{-1}$ IBA as well as half MS salt strength with the use of glucose and 1 $mg.l^{-1}$ IBA. However, the greatest number of roots per explant was obtained when half MS salt strength was used with Sucrose and 1$mg.l^{-1}$ IBA which gave 11.33 roots, while the least number of roots was achieved when full MS salt strength was used with glucose and 0 and 1 $mg.l^{-1}$ IBA which was 1.33 roots. Moreover, the longest roots were achieved when full MS salt strength was used with sucrose and 0 $mg.l^{-1}$ IBA by giving 3.53 cm, while the shortest roots were acquired when full MS salt strength with glucose and 1 $mg.l^{-1}$ IBA

was used by giving 0.37 cm. The woody nature of the scarlet firethorn, a high auxin concentration required for root induction is most likely due to this (Dirr and Heuser, 2009). The auxin kinds and concentrations, as well as the macronutrients in the culture media, had a substantial impact on the root development of *P. coccinea*. Root proliferation was facilitated by the culture medium's lower macronutrient content., according to the findings (Chao *et al*, 2017).

Table (2): Effect of carbon source, MS salt strength and IBA concentrations on rooting stage of *Pyracantha coccinea* explants after six weeks in culture

MS salt strength	Sugars	IBA	Rooting percentage (%)	No: roots/ explant	Roots ength (cm)
Full	Sucrose	0.0	67	3.33 c	3.53 a
		0.5	100	1.67	1.87 c
		1.0	83	4.67 b	2.00 c
	Glucose	0.0	67	1.33 d	0.43 d
		0.5	67	3.00 c	1.43 c
		1.0	67	1.33 d	0.37 d
Half	Sucrose	0.0	83	4.67 b	2.73 b
		0.5	100	3.33 c	1.83 c
		1.0	83	11.33 a	3.37 a
	Glucose	0.0	83	3.33 c	1.83 c
		0.5	83	4.67 b	2.33 b
		1.0	100	5.33 b	1.83 c

*Data followed by the same letters on the same column are non-significantly different according to Duncan multiple range test at 0.05 level.

At acclimatization stage, *Pyracantha coccinea* plantlets were successfully acclimatized with a survival rate of 80% in sand, 95 in peatmoss and 85% in sand + peatmoss. The plantlets can be transported and adapted to the ambient environment once they have been rooted. This micropropagation approach has the potential to produce large quantities of elite genotypes or modern strains of *P. coccinea*.

Many woody plants including ornamentals are resistant to vegetative proliferation using traditional procedures. As a result, an micropropagation approach has been created, however the needs of each species could be highly different. methodology with varied problems. The concentration of growth regulators, carbon sources, and the nutritional salt level of the media each have an effect on *P. coccinea* shoot proliferation, shoot multiplication and rooting. 1 $mg.l^{-1}$ BA was employed to initiate shoot growth and proliferation in this study for *P. coccinea*, and it has been noted that this concentration acceptable for the establishment of microshoots in several species, including *Pyracantha coccinea* M. Roem (Chao *et al*., 2017), Siberian elm (Ulmus pumila) (Cheng and Shi, 1995).

Conclusions

The use of benzyl adenine in the stage of initiation and multiplication for shoot proliferation is important to obtain a high branching of plants because it works to eliminate the apical dominance, and the auxins (IBA) work to stimulate the formation of roots in the plant, and the low strength of the salts of the food medium helps to stimulate the growth of roots due to the increase in the ratio of carbon to nitrogen, and that sucrose is the best source of carbon used in the propagation and rooting of *Pyracantha coccinea* which can be rapidly propagated by plant tissue culture. This technique is easy and used for plants that have problems with traditional methods.

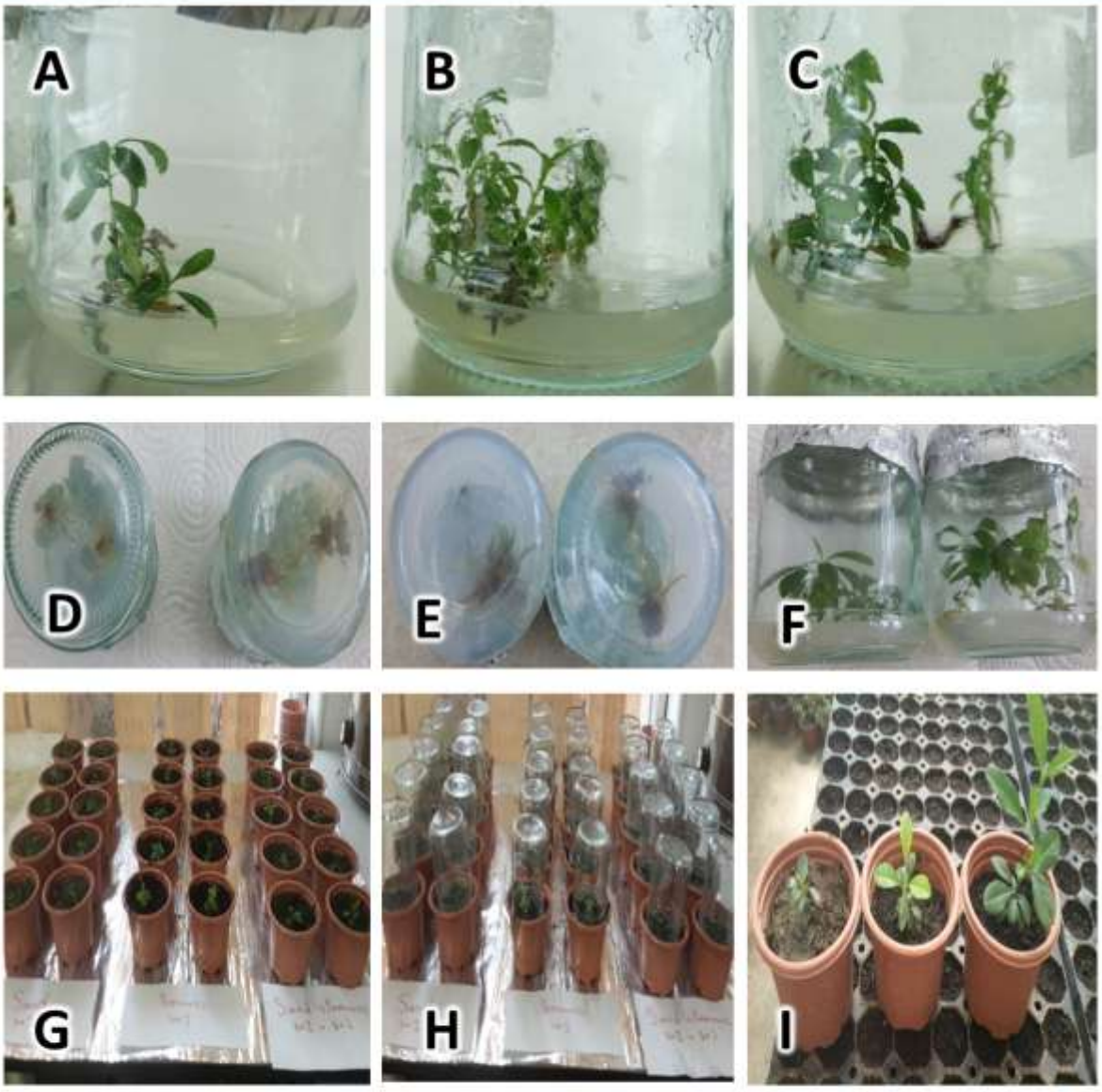

Figure 1. ***In vitro*** **propagation stages of** ***Pyracantha coccinea*** **explants grown on MS medium:**

A: Explants initially inoculated on the medium. B: Six weeks growing explants on shoot multiplication medium when 2.5 mg.l^{-1} BA was used. C: Six weeks growing explants on shoot multiplication medium when 1.5 mg.l^{-1} Kin was used. D: Well rooted shoots at root formation stage. E & F: The best rooting treatment (IBA 1.0 mg.1-1 and sucrose). G: The beginning of acclimatization stage in sand, peatmoss and sand + peatmoss. H&I: Well acclimatized plantlets under greenhouse conditions.

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