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Original Research

Rapid *in vitro* regeneration of *Geranium robertianum* - a valuable medicinal herb

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ABSTRACT:

The present study highlights a rapid and simple micropropagation of *Geranium robertianum*. Micro shoots were regenerated from shoot tips and nodal explants of *in vitro* seedlings directly without the intervening callus phase. *In vitro* shoots were successfully proliferated from those explants of *G. robertianum* cultured on MS medium fortified with Naphthalene Acetic Acid (NAA) and of N6-Benzylaminopurine (BAP). Results showed that proliferated shoots from shoot tips reached the highest mean (3.715); compared with those from nodal explants (3.015). The application of an adjuvant, adenine sulphate (80 $mg.L^{-1}$) in the optimal medium led to increase the mean shoot number per shoot tip of 4.60 shoots.

Keywords:
Geranium robertianum, Medicinal plant, *in vitro* multiplication.

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INTRODUCTION

G. robertianum L. (*Geraniaceae*), is one of the garden plants, is known as Red Robin, Herb Robert and Storks bill. This plant is native to Europe, Asia, North Africa and North America (Elobieta *et al*., 2017). It grows as a procumbent to biennial or erect annual plant, with the strong scent, up to fifty centimeter high, producing small, pink, five petal led flowers (8–14 mm in diameter) from April until the autumn. It has deeply dissected leaves, triplex to palmate, and the stems often reddish; at the end of the flowering season, the leaves also turn red (Elobieta *et al*., 2017).

Scientific classification

Kingdom : Plantae
Clade : Eudicots
Clade : Rosids
Order : Geraniales
Family : Geraniaceae
Genus : *Geranium*
Species : *G. robertianum*

In traditional herbalism, Red Robin was used for the treatment of wounds, ulcers and diarrhea, and also as a remedy for toothache and nosebleeds. The active compounds identified in *G. robertianum* are phenolic acids, tannins, flavonoids, essential oil and vitamins (A, C). Plants have the ability to produce chemical compounds that help them defend against attack from a wide variety of predators, such as herbivorous mammals, insects and fungi (Igwenyi and Elekwa, 2014; Lai and Roy, 2004).

Earlier *in vitro* propagation through meristem tip culture (Jugulam *et al*., 2001), direct shoot regeneration (Zuraida *et al*., 2013), stem explants and nodal culture (Gupta *et al*., 2002; Charlwood and Charlwood, 1991; Rabuma, 2017), leaf and petiole explants (Gauri *et al*., 2008; Ghanem *et al*., 2008) and callus culture (Ghanem *et al*., 2005) were reported in Geraniaceae family plants. The present investigation aims at developing a productive protocol, which can be used for rapid mass propagation of *Geranium robertianum* to equate the pharmaceutical demand.

MATERIALS AND METHODS

Plant materials and sterilization

This study was conducted from September 2017 to February 2018 in the Laboratory of Plant Tissue Culture, Department of Plant Genetic Resources, Ministry of Agriculture, Baghdad. *Geranium robertianum* seeds were collected from the plants growing wild at Kurdistan region, North of Iraq by plant genetic resources team, Seed Testing and Certification Directorate (STCD), Ministry of Agriculture, Iraq. The seeds were washed thoroughly under a fast running tap water and later rinsed with distilled water, then were transferred to laminar air flow chamber and finally surface sterilized with 6% of commercial Clorox containing a drop of tween-20 for 5 min. After three rinses with sterile water, the sterilized seed cultured in test tubes (25×100 mm) containing MS medium (Murashige and Skoog, 1962). Cultures were checked up ordinarily for contaminations and those presented obvious infection.

Culture conditions and shoot multiplication

Seeds were germinated after 35 days of culture and the *in vitro* seedlings were used as a source of explants. For induction and multiplication of shoots, shoot tips (0.2 cm) and nodes (1cm) were cut and cultured on solidified MS media, supplemented with growth regulators (BAP), used independently, at different concentrations (0.0, 1.0, 2.0 and 3.0) $mg.L^{-1}$ and in combination with NAA at different concentrations (0.0, 0.1, 0.2 and 0.4) $mg.L^{-1}$. The effect of adenine sulphate was tested by adding it to MS medium at different concentrations (0.0, 40.0, 80.0, 120.0) $mg.L^{-1}$, plus (2.0 $mg.L^{-1}$ BAP and 0.1 $mg.L^{-1}$ NAA) for shoot tip multiplication medium and (1.0 $mg.L^{-1}$ BAP and 0.2 $mg.L^{-1}$ NAA) for nodal multiplication medium (Afshan *et al*., 2014).

After four weeks, the responding explants were transferred to the same medium containing various con-

Table 1. Effect of BAP on shoot regeneration from grown shoot tips and nodal explants on MS medium after eight weeks of culture

S. No	Explant	Concentration of BAP (mg.L^{-1})				L.S.D (=0.05)
		0.000	1.000	2.000	3.000	
1	Shoot tip (Mean shoot number)	0.000	0. 600	3.460	1.450	0.541
2	Node (Mean shoot number)	0.000	0.911	1.956	1.072	0.541

Table 2. Effect of BAP-NAA in combination on shoot regeneration from grown shoot tips on MS medium after eight weeks of culture

S. No	BAP (mg.L^{-1}) / NAA (mg.L^{-1})	0.000	1.000	2.000	3.000	Mean
1	0.000	0.000	0.682	3.462	1.454	1.399
2	0.100	0.000	1.258	3.715	0.619	1.398
3	0.200	0.000	1.976	2.312	0.664	1.238
4	0.400	0.000	0.786	1.494	0.000	0.570
	Mean	0.000	1.175	2.746	0.684	
	LSD 0.05	BA=0.2392	NAA=0.261	NAA* BA=0.505		

centrations and combinations of growth regulators after harvesting the regenerated shoots. The number of regenerated shoots was recorded after eight weeks of incubation. All the cultures were incubated in a plant growth room at a temperature of 21±1ºC and with 16/8 h light/ dark cycles under cool-white fluorescent lamps (1000 lux).

Experimental design and data analysis

The experimental design used in this study in all the experiments was a Completely Randomized Design (CRD). Analysis of variance was conducted using General Linear Model's procedure of the Statistical Analysis System version 9.2. Mean comparisons were made using Least Significant Difference (LSD) (Steel *et al*., 1997) test. Each treatment was done in 10 replications. SPSS 16 software was used for statistically analysis of the data (Snell and Simpson, 1991).

RESULTS AND DISCUSSION

Explants cultured on MS basal medium served

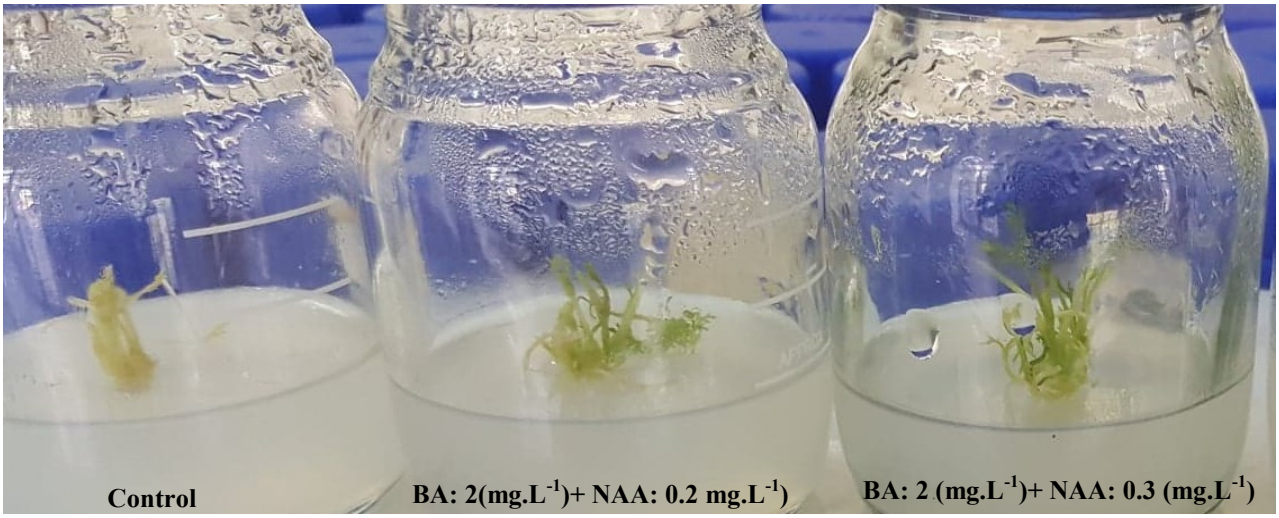

Figure 1. Effect of BAP-NAA in combination on shoot regeneration from grown nodes on MS medium after eight weeks of culture

Table 3. Effect of BAP-NAA in combination on shoot regeneration from grown nodes on MS medium after eight weeks of culture

S. No	BAP (mg.L^{-1}) / NAA (mg.L^{-1})	0.000	1.000	2.000	3.000	Mean
1	0.000	0.000	0.985	1.956	1.072	0.990
2	0.100	0.000	1.434	0.522	0.141	0.524
3	0.200	0.000	3.015	1.390	0.459	1.216
4	0.400	0.000	0.786	0.318	0.000	0.276
	Mean	0.000	1.536	1.046	0.418	
	LSD 0.05	BA=0.2728	NAA=0.1942	NAA* BA=0.4283		

Table 4. Shoot initiation from shoot tip and nodal explants of *G. robertianum* as affected by adding adenine sulphate in the optimal medium

S. No	Explant	Adenine sulphate concentrations				L.S.D (=0.05)
		0.000	1.000	2.000	3.000	
1	Shoot tip (Mean shoot number)	3.60	3.80	4.60	1.60	1.264
2	Node (Mean shoot number)	3.00	2.20	2.40	1.00	1.572

as control, did not enhance shoot proliferation and observations were recorded on the growth response of the explants to the varying growth regulator combinations.

Table 1 showed that the shoot tip and nodal explants get proliferated shoots of incubation in different BAP concentrations tried, which is similar with the reports of Charlwood and Charlwood (1991); Zuraida *et al*. (2013) and Rabuma (2017) where they reported significant shoot regeneration from the shoot tip and nodal explants, the highest mean shoot regeneration for both explants (3.460 and 1.956) respectively, were observed on MS medium supplemented with 2 mg.L^{-1} BAP.

The relative effectiveness of various cytokinins for multiple shoot formation revealed the order of effectiveness as BAP to be the most effective plant growth regulator compared to other cytokinins due to the inter-

Figure 2. Shoot multiplication in MS medium fortified with 2.0 mg.L^{-1} BAP, 0.1 mg.L^{-1} NAA and adenine sulphate (80 mg.L^{-1})

nal structure and number of bonds in its side chain as well as the presence of benzyl ring, making it the greater cytokinins that affect the division and growth (Staden and Crouch, 1996).

The effect of combined treatments of cytokinin along with auxin was evaluated for shoot multiplication rate for both explants, by taking the concentrations of BAP (1, 2 and 3 $mg.L^{-1}$) in combination with different concentrations (0.1, 0.2 and 0.4 $mg.L^{-1}$) of auxin (NAA) (Tables 2, 3 and Figure 1).

Shoot tips and nodal explants germinated from *in vitro Geranium robertianum* seedlings were cultured on MS media containing BAP-NAA combinations, Shoot initiation was recorded after eight weeks of cultivation. The best combination of growth regulators producing shoots per shoot tip (3.715) was 2 $mg.L^{-1}$ BAP and 0.l $mg.L^{-1}$ NAA Table 2. While the best combination for producing shoots per node (3.015) was 1 $mg.L^{-1}$ BA and 0.2 $mg.L^{-1}$ NAA Table 3. No shoots were observed when both explants cultured on MS medium was free of BAP.

Our results approximately agreed with Gupta *et al*. (2002) and Ghanem *et al*. (2008) and are ensured by Skoog and Miller (1957) who found that relatively high cytokinin to auxin ratio brought about shoot formation in *Nicotiana tabacum*.

The highest mean shoot multiplication per shoot tip was recorded in MS supplemented with 2.0 $mg.L^{-1}$ BAP and 0.1 $mg.L^{-1}$ NAA and adenine sulphate (80 $mg.L^{-1}$) Table 4 and Figure 2. Data in this Table 4 (In case of shoot tip) showed that using 80 $mg.L^{-1}$ adenine sulphate recorded the best result for producing shoots (4.60). Similarly, Bantawa *et al*. (2009) reported that the addition of adenine sulphate along with other growth regulators was the most effective in inducing shoot multiplication. According to Murashige (1974), adenine in the form of adenine sulphate can activate cell growth and highly enhance the shoot formation. It is apparent from the results that adenine sulphate was found to reinforce the effect of other PGRs as implied by the enhancement in shoot proliferation.

CONCLUSION

Our study highlights the recent achievements for the rapid micropropagation of *Geranium robertianum* in Iraq, the shoot multiplication of this species depended upon the treatment that is tested. The heist number of proliferated shoots per shoot tip was obtained on the MS media fortified with (2.0 $mg.L^{-1}$ BAP and 0.1 $mg.L^{-1}$ NAA) in combination with adenine sulphate (80 $mg.L^{-1}$). In conclusion, here we record a fast protocol for *G. robertianum* regeneration from the nodes and shoot tips that can support a reliable *in vitro* culture system for commercial propagation.

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