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Influence of phytohormones on *in vitro* callus induction of *Glycyrrhiza glabra* L. using nodal segments

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Abstract
The nodal segment explants of *Glycyrrhiza glabra* L. were utilized to develop an efficient *in vitro* callus induction protocol. Full strength MS medium was tested with NAA and 2, 4-D alone and BAP with NAA and 2, 4-D respectively. Concentrations 1.0-5.0 mgL^{-1} of NAA and 2, 4-D were used alone and BAP (3.0 mgL^{-1}) with 1.0-2.0 mgL^{-1} of NAA and 2, 4-D concentrations were used for the incubation of the nodal segments respectively. Amongst all, the combined concentration of BAP (3.0 mgL^{-1}) with NAA (3.0 mgL-^{1}) was given superior results as 100 % of callus formation with 13.07 ± 0.39 average days taken to initiate callus. Today, Glycyrrhiza species became endangered because of overexploitation and demolition of a home as it contains many valuable remedial properties. Hence the present investigation was undertaken to establish an in vitro shoot formation protocol to preserve the important medicinal plant *Glycyrrhiza glabra* L.

Keywords: *in vitro*, induction, protocol, overexploitation, demolition, remedial

1. Introduction
Glycyrrhiza glabra L. commonly known as liquorice is a semi bushy or grassy type perennial plant belongs to the family Leguminosae. It is native to the Central, Southwest Asia and Mediterranean regions. Since prehistoric times, roots and rhizomes of this species have been used globally as a natural sweetener as well as an important drug in oriental medicine [4]. Glycyrrhizin is an active principle chemical constituent present in the dried rhizomes and roots of this shrub which is used in pharmaceutical and some candies for sweetness and flavor [16] as well as also utilized in confectionery and tobacco industries [13]. Many pharmaceutical industries concentrate on this species as it contains many therapeutic properties such as antipyretic, antiallergy, antiulcer, antidiabetic, anti-inflammatory, anticarcinogenesis and laxative [1, 16].
This species is propagated by seed which is a traditional method of propagation and used extensively in local areas. The germination percentage of the seeds is very poor due to the presence of its hard seed coat which restricts its multiplication [14]. Moreover, seed availability, seed dormancy and adverse atmosphere are the main constraints in using the seed for the propagation of liquorice [2, 5]. The production of natural species of *Glycyrrhiza* is harshly reduced because of the excessive and disastrous consumption. So, today it became endangered species and has been placed in a red data book, therefore, it needs preservation. The demand of the medicinal and confectionery industries for raw material of this species increasing constantly as it contains valuable medicinal properties.
So, keeping in mind all such facts of this species, the current experiment was carried out to establish an efficient protocol of *in vitro* callus induction that can be helpful in favor of rapid multiplication for conservation as well as meet the increasing global demands.

2. Material and Methods
2.1 Explant Material
Stolon cuttings of newly emerged were obtained from Dhanwantary Udyan, Mahatma Phule Agricultural University, Rahuri, District, Ahmednagar in Maharashtra State. Primarily, these cuttings were preserved appropriately under shade net house and after a week they were cultivated in open field at Shikshan Maharshi Dnyandeo Mohekar Mahavidyalaya, Kalamb. After six months, they were developed into well mature plants. From such plants, the nodal segments were cut and used as explants for *in vitro* callus induction.

2.2 Method of *in vitro* culture
In the current experiment, MS [9] medium with full strength was used which contains 30 gL^{-1} sucrose and 8 gL^{-1} agar supplemented with 1.0, 2.0, 3.0, 4.0 and 5.0 mgL^{-1} concentrations of 1-naphthalene acetic acid (NAA), and 2, 4-Dichlorophenoxyacetic acid (2, 4-D) alone and 3.0 mgL^{-1} 6-Benzyl amino purine (BAP) in combination with 2.0 and 3.0 mgL^{-1} concentrations of NAA and 2, 4-D respectively. With the use of 1 N NaOH or 1 N HCl, the pH of the medium was adjusted to 5.8 and kept for autoclaving at 121°C for 20 min. The explants such as the nodal segments were excised from a healthy mature plant and cut into 1-2 cm in size. These explants were disinfected with water detergent under running tap water to eradicate dirt particles. Under the aseptic atmosphere, these explants were treated with 0.1% (w/v) mercuric chloride for 3-4 minutes. The explants were then completely cleaned 4-5 times with germ-free double distilled water to abolish the traces of mercuric chloride. The end of the explants was cut and finally inoculated on MS medium. The cultures were kept in a culture room at a temperature of 25±2ºC and a 16-h photoperiod (intensity of 4000 lux). The experiment was

repeated three times using 15 replicates with one explant per culture bottle. After four weeks of inoculation, the visual observations such as callus growth, number of days to initiate callus and percentage of callus cultures were noted.

3. Statistical analysis

All the remarks of the present work were noted at the interval gap. They were primarily estimated with their Mean ± SE. Data were analyzed with the use of One Way ANOVA and Duncan Multiple Range Test (DMRT). For the statistical investigation, Statistical Package for Social Science (SPSS, version 11.5) software at a 5% level ($p<0.05$) was applied.

4. Results and Discussion

4.1 Influence of NAA and 2, 4-D on callus induction

In the current study, the nodal segments incubated on MS medium fortified with 1.0, 2.0, 3.0, 4.0 and 5.0 mgL^{-1} concentrations of NAA and 2, 4-D alone respectively [table 1]. Amongst all, NAA (3.0 mgL^{-1}) concentration was found most favorable as it was given 90.53±0.37 % of callus induction and mean time to induce callus was 14.33±0.21 days. The induced callus was friable and creamy, brown shown excellent growth [table 1 and figure A]. These results are inconsistent with the findings of Gita Rani and Grover (1999) [6] who observed the best callusing (92%) of *W. sominifera* on the medium supplemented with 2, 4-D and KN.

4.2 The combined effect of BAP with NAA and 2, 4 -D on shoot induction

In the current experiment the nodal segments inserted on MS medium supplemented with BAP (3.0 mgL^{-1}) concentration with the combination of NAA and 2,4-D (2.0-3.0 mgL^{-1}) concentrations respectively [table 2]. Amongst all, BAP (3.0 mgL^{-1}) concentration combined with NAA (3.0 mgL^{-1}) concentration was shown most superior results as it was given 100% of callus induction and mean time to induce callus was 13.07±0.39 days. The induced callus was friable with greenish creamy and shown excellent callus growth [table 2 and figure C]. Similar findings have also been recorded in liquorice by Patel and Shah (2007) [11] for nodal segment culture. Dewir *et al.* (2010) [3] in *Withania somnifera* plant also noted that the combination of BAP along with auxin showed good callus growth rather than BAP alone. Thus the results of the current study verified the previous remarks and recommend that the nodal segment explants and the combination of BAP with NAA concentration could be efficiently used for *in vitro* callus induction.

Table 1: Effect of NAA and 2, 4-D on callus formation using nodal segments

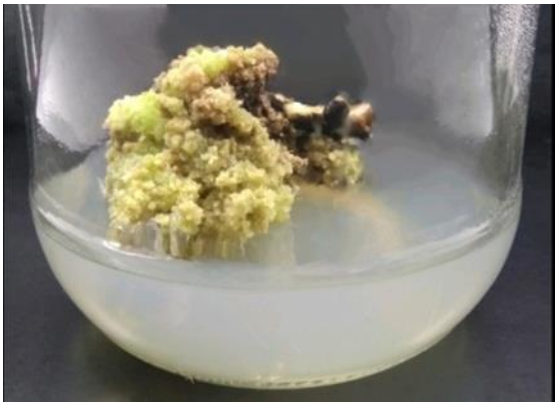
Explant	Plant Growth Regulators (mgL^{-1})		No. of days taken to induce callus (Mean ± SE)	% Of cultures initiating callus (Mean ± SE)	*In vitro* visual remarks		
	NAA	2.4-D			Growth	Texture	Color
Nodal segment	1	-	17.67 ± 0.42	70.67 ± 0.45	C^{+++}	FF	GC
	2	-	15.27 ± 0.38	70.75 ± 0.44	C^{++++}	F	GW
	3	-	14.33 ± 0.21	90.53 ± 0.37	C^{++++}	F	CB
	4	-	18.13 ± 0.53	60.93 ± 0.52	C^{++}	F	CB
	5	-	20.53 ± 0.55	50.47 ± 0.61	C^{+}	H	CBW
	-	1	18.20 ± 0.51	70.13 ± 0.60	C^{+++}	FF	GC
	-	2	17.40 ± 0.46	70.20 ± 0.52	C^{++++}	F	GW
	-	3	15.67 ± 0.31	80.67 ± 0.46	C^{++++}	F	CB
	-	4	19.33 ± 0.55	60.33 ± 0.62	C^{++}	F	CB
	-	5	20.60 ± 0.57	60.07 ± 0.68	C^{+}	H	CBW

Growth of callus : (C^{+}) Very poor growth of callus; (C^{++}) Poor growth of callus; (C^{+++}) Good growth of callus; (C^{++++}) Very Good growth of callus; (C^{++++}) Excellent growth of callus Color of Callus : (GC) Greenish, creamy; (GW) Greenish, white; (GY) Greenish, yellow; (CB) Creamy, browning; (CBW) Creamy, browning with slight white Texture of Callus : (F) Friable; (FF) Firm and friable; (SFF) Slightly firm and friable; (L) Loose; (H) Hard

Table 2: The combined effect of BAP (3 mgL^{-1}) with NAA and 2, 4-D on callus formation using nodal segments

Explant	Plant growth regulators (mgL^{-1})		No. of days taken to induce callus (Mean ± SE)	% Of cultures initiating callus (Mean ± SE)	*In vitro* visual remarks		
	NAA	2,4-D			Growth	Texture	Color
Nodal segment	3	-	13.07 ± 0.39	100 ± 0.00	C^{++++}	F	GC
	2	-	17.47 ± 0.60	90.13 ± 0.09	C^{++++}	F	CB
	-	3	15.20 ± 0.42	90.33 ± 0.33	C^{++++}	F	GC
	-	2	19.27 ± 0.64	80.67 ± 0.45	C^{++++}	L	Y

Growth of callus : (C^{+}) Very poor growth of callus; (C^{++}) Poor growth of callus; (C^{+++}) Good growth of callus; (C^{++++}) Very Good growth of callus; (C^{++++}) Excellent growth of callus Color of Callus : (GC) Greenish, creamy; (GW) Greenish, white; (GY) Greenish, yellow; (CB) Creamy, browning; (CBW) Creamy, browning with slight white Texture of Callus : (F) Friable; (FF) Firm and friable; (SFF) Slightly firm and friable; (L) Loose; (H) Hard



(A)

(B)

(C)

Fig 1: Showing figure (A) Shoot formation on nodal segment using NAA (3 mgL^{-1}) concentration, figure (B) Shoot formation on nodal segment using 2, 4-D (3 mgL^{-1}) concentration, figure (C) Shoot formation on nodal segment using BAP (3 mgL^{-1}) with NAA (3 mgL^{-1})

5. Conclusion

In the current experiment, *in vitro* callus induction was achieved on MS medium fortified with different concentrations of auxins alone by using the nodal segments as explants. Most appropriate concentrations were found to be 3.0 mgL^{-1} of NAA when supplemented alone in MS medium. Callus induction was also aimed at using the nodal segment supplemented on MS medium from cytokinin (BAP) combined with auxins (NAA and 2, 4-D) respectively. The efficient concentrations were found to be 3.0 mgL^{-1} of BAP combined with 3.0 mgL^{-1} of NAA when supplemented in MS medium. The lower concentrations of auxin alone or combination with cytokinin were given maximum results than higher concentrations. Higher concentrations were shown a hazardous effect on shoots. The established an *in vitro* callus induction protocol has the prospective to rejuvenate plants on a major scale in a short period which will be beneficial in the future for preservation as well as meets the requirement of the world.

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