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Callus induction and shoot regeneration in *Ducrosia anethifolia* an important threatened medicinal plant

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ABSTRACT

Ducrosia Anethifolia is a threatened medicinal herb belongs to the family Apiaceae. This important medicinal plant is a native medicinal plant in Iran and has a restricted distribution in the world. It is used to treat of headache and backache in folk medicine. To callus induction, Leaf and lateral bud were used as explants and the Culture media was MS medium supplemented with 3% w/v sucrose, 0.7% w/v agar and 1gl^{-1} myo-inositol at various concentration of cytokinins and auxins. After Five weeks, the calluses were transferred into regeneration medium that included MS medium supplemented with the combination of BA(0.5, 1 and 2 m gl^{-1}) and NAA(0, and 0.5 mgl^{-1}). The callus percentage, callus size and fresh weight, shoot length and number of shoots per explants were recorded. The analysis of variance showed that explants and media had significant effects on callus induction. The results revealed that the highest percentage (86.7%) of callus induction was created on MS media containing 2mg/l NAA and 1mg/l BA. On the other hand, lateral bud explants gave higher values in callus inducing percentage, fresh weight and size compared to leaf explants. In the case of plant regeneration, results indicated that the longest shoot length (21.6 mm) was exhibited for explants cultured on MS-medium containing 0.5mg/l BA and the highest number of shoots per explants(5.6) was obtained from callus grown on the medium supplemented with 2mg/l BA.

Key word: *Ducrosia anethifolia*, callus induction, regeneration, explants, media

Introduction

Ducrosia anethifolia is a threatened medicinal plant belongs to the family Apiaceae. This important medicinal herb with a height of about 10 to 30 cm has a restricted distribution, mainly in Iran, Iraq, Pakistan and Afghanistan [9]. The essential oil of the aerial parts of this herb contain some phytochemical compounds especially an antimycobacterial coumarin [17]. The biochemical studies revealed that the seed oil of *Ducrosia anethifolia* contains about 58.8% petroselinic acid with amounts of oleic, linoleic, linolenic and palmitic acids [12]. This high percentage of petroselinic acid makes this herb as a suitable raw material source in soap and chemical industries. Several reports show that this herb is used to treat of headache and backache in folk medicine and also to relax the mind and body and induce a peaceful sleep [7]. Climatic changes from the last decades, soils and water pollution are the main reasons for threatening many herbs. So it is necessary to find efficient procedures for conservation of these germplasms [2]. One of the best possible methods of protection of endangered plants is micropropagation. On the other hand, in vitro culture techniques could represent a very useful tool for mass propagation of superior stock plants as well as genetic improvement. The in vitro propagation of medicinal plants provide a source of uniform and sterile plant material for mass multiplication and germplasm conservation of endangered and threatened medicinal plants [1,18]. To our knowledge, there are a few reports on invitro propagation of *Ducrosia anethifolia.* The aim of this study was to study the effect of different concentration of growth regulators on callus induction from leaf and lateral bud explants and optimize a protocol for plant regeneration of induced calli.

Materials and methods

Explant excision and Media preparation:

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The mature seed of *Ducrosia anethifolia* were obtained from the institute of medicinal plants(IMP), Tehran,Iran. The soaked seeds were sterilized by 70% ethyl alcohol for 1 min. This was followed by surface sterilization in 2.5% sodium hypochlorite solution for 8 min followed by 3 rinses in sterile water. Then sterile seeds were planted on ½ MS (murashige and skoog 1962) medium supplemented with $30gl^{-1}$ sucrose and 7 gl^{-1} agar. The cultures were incubated at growth chamber at 23± 2 ^{0}C with 16/8 h illumination periods. After seed germination, leaf and lateral bud were taken from the plantlets to use as explants for callus induction. The Culture media was prepared using Murashige and Skoog basal medium salts supplemented with 3% w/v sucrose, 0.7% w/v agar and $1gl^{-1}$ myo-inositol at various concentration of cytokinins and auxins. The cytokinins used were benzyladenine (BA) and kinetin (KIN), while naphthalene acetic acid (NAA) and 2,4- dichlorophenyoxy acetic acid (2,4-D) were the auxin used (Table 1). All growth regulators were added before autoclaving and pH was adjusted to 5.7 ± 0.1.

Table 1: Structure of testing media for callus induction of *Ducrosia Anethifolia.*

Media code	Hormone combination			
	2,4-D (mg/l)	NAA (mg/l)	BA (mg/l)	Kin (mg/l)
1	-	1	0.5	-
2	-	2	2	-
3	2	-	-	1
4	-	2	1	-
5	2	-	-	2
6	1	-	-	0.5

Callus induction and Plant regeneration:

Five segments of each explants(leaf and lateral bud) were cultured into MS medium that supplemented with the combination of cytokinin and auxin(Table 1). The cultures were incubated in growth chamber at 25± 1 ^{0}C with 16/8 h illumination periods. After five weeks of incubation, callus percentage, callus size and fresh weight were measured (Fig 1).

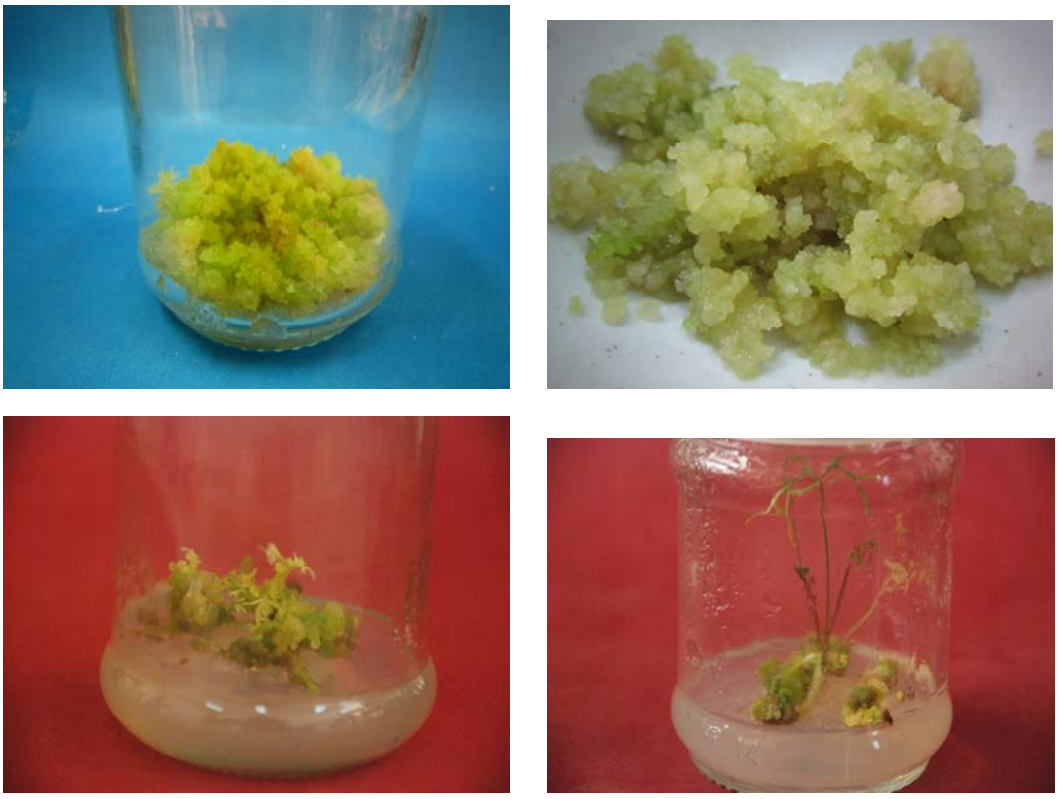

Fig. 1: Explants were cultured in various combinations of growth regulators to induce callus. After callus induction, The calluses were transferred to regeneration culture media containing various concentrations of BA and NAA to produce shoots.

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Five-week-old calluses were transferred into regeneration medium that included MS medium supplemented with the combination of BA(0.5, 1 and 2 m gl^{-1}) and NAA(0 and 0.5 mgl^{-1}). The cultures incubated in growth room at 25± 1 ^{0}C with 16/8 h illumination periods. After five weeks of incubation, shoot length and number of shoots per explant were recorded.

Statistical analysis:

The data were statistically analyzed as a factorial experiment based on completely randomized design (CRD) with three replicates. Comparisons among means were made using Duncan's multiple reng test(DMRT) at 5% level of probability. The data were analyzed using Spss software.

Results and Discussion

Statistical analysis of Data showed that explants and media had significant effects on callus induction(Table 2). Duncan's multiple rang test revealed that the greatest percentage (86.7%) of callus induction was created on MS media containing 2mg/l NAA and 1mg/l BA. The lowest callus inducing percentage were obtain by using 1mg/l 2,4-D in combination with 0.5 mg/l Kinetin. The results also indicated that the highest callus fresh weight and size were obtained by MS media supplemented with 2mg/l NAA and 1mg/l BA. On the other hand, means presented in Table 3 revealed that lateral bud explants gave higher values in callus inducing percentage, fresh weight and size compared to leaf explants. This result suggested a positive relationship between callus induction and callus weight and size.

Table 2: Analysis of variance for the effect of explants and mediums and their interaction on callus induction (%), callus fresh weight(gr), and callus size (mm).

Source of variation	Mean of squares			
	df	Callus induction	Callus fresh weight	Callus size
Explant	1	900*	0.052**	5.7**
Medium	5	1211.1**	0.128**	15.1**
Explant×medium	5	20 $^{n.s}$	0.001 $^{n.s}$	0.346$^{n.s}$
Erorr	24	155.5	0.001	0.17

**: Highly Significant at 0.01 probability level, *: Significant at 0.05 probability level and n.s: Not significant.

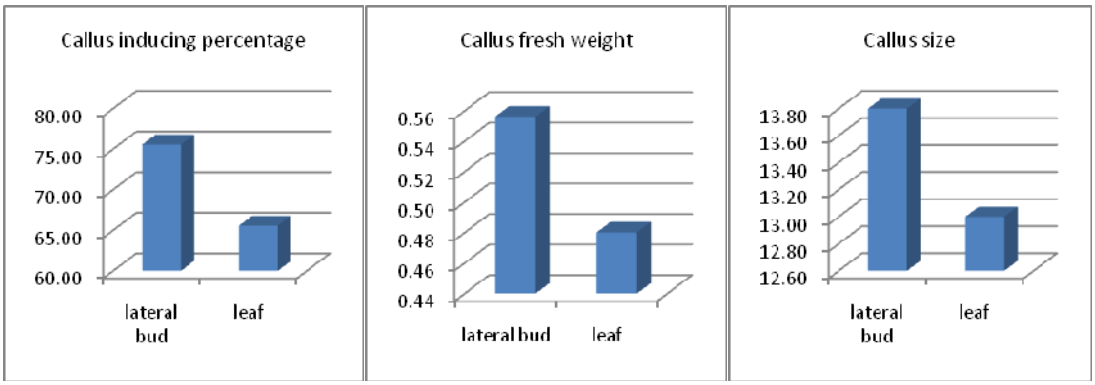
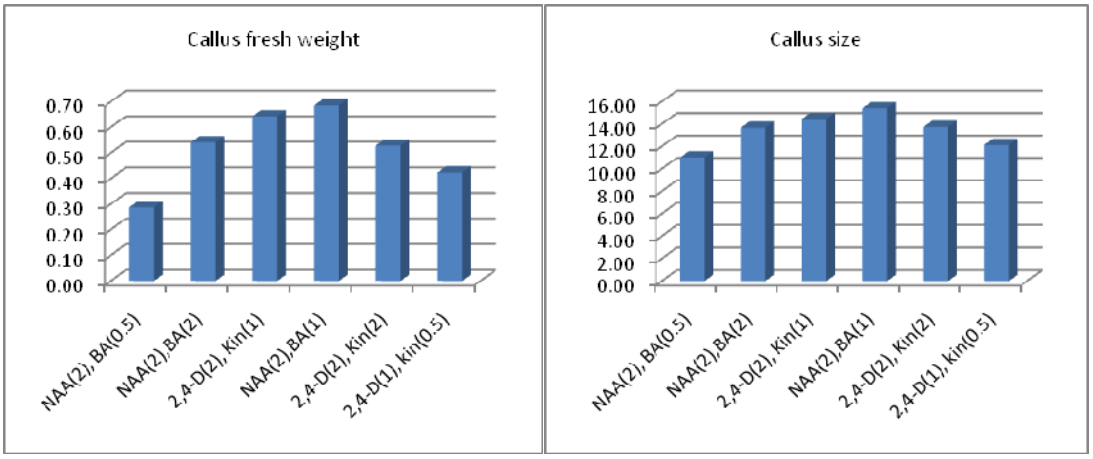


Fig. 2: The influence of explants on callus inducing (%),Callus fresh weight (gr) and callus size(mm).



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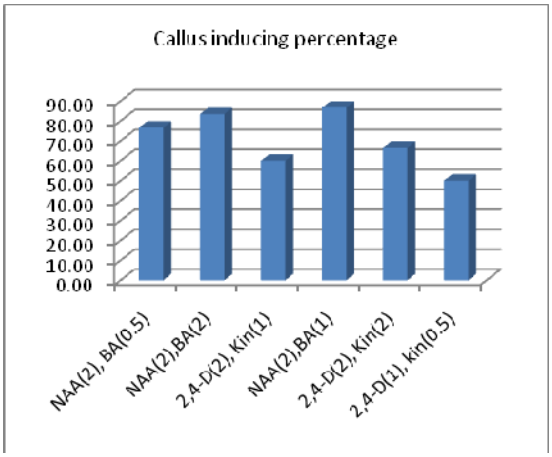

Fig. 3: The influence of media combination on callus inducing (%),Callus fresh weight (gr) and callus size(mm).

The results revealed that in the case of callus induction, the use of NAA as auxin source gave higher values in comparison with 2,4-D and with increasing NAA, the callus induction, callus size and fresh weight increased. Increase in concentration of NAA (as auxin source) in combination with BA (as cytokinin source) in the media had a stimulatory effect on callus induction ability of both explants. These findings about the positive influence of NAA on callus induction are in agreement with those reported by Karimi *et al*. [11] on *Ducrosia flabellifolia*; Irvani *et al*. [8] on *Dorem ammoniacum*; Barakat *et al*. [4] on *Gypsophila paniculata* and Bernard *et al*. [5] on *Ferula gummosa*.

Table 3: Means of callus induction (%),Callus fresh weight (gr) and callus size(mm) as affected by medium and explant.

Factor level				
explant	medium	Callusing percentage	Callus fresh weight	Callus size
1	1	80.00 ab	0.32 d	11.43 e
	2	86.67 ab	0.53 b	13.83 c
	3	66.67 bc	0.67 a	14.73 b
	4	93.33 a	0.74 a	16.23 a
	5	73.33 ab	0.56 b	13.97 bc
	6	53.33 c	0.46 c	12.6 d
2	1	73.33 ab	0.25 d	10.6 d
	2	80.00 a	0.51 b	13.5 b
	3	53.33 ab	0.62 a	14.17 ab
	4	80.00 a	0.63 a	14.57 a
	5	60.00 ab	0.50 b	13.5 b
	6	46.67 b	0.38 c	11.67 c

*Means followed by the same letter(s) are not significant, different at 0.05 level of probability.

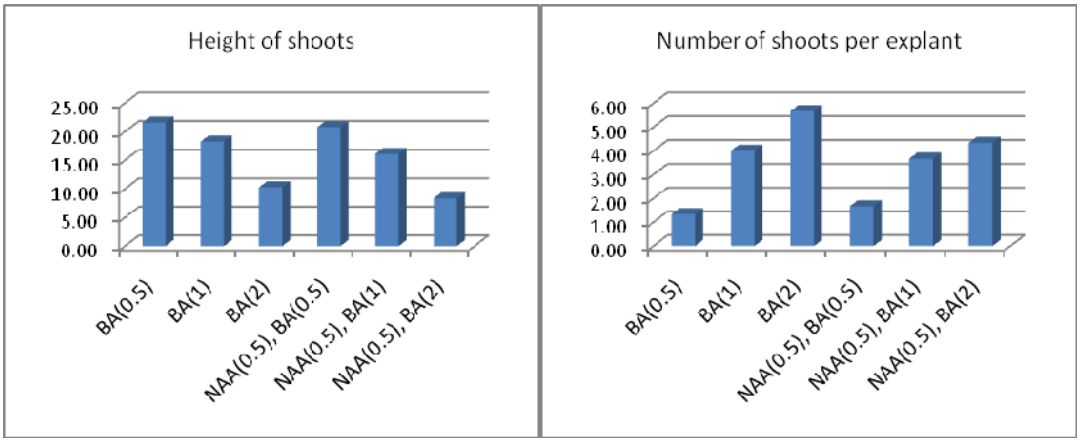

Fig. 4: The influence of media combination on Number of shoots per explants and Height of shoots (mm).

In the case of plant regeneration, results indicated that different concentrations of the cytokinin BA and the auxin NAA tested in this study had a significant effect on the regeneration of plantlets. The longest shoot length (21.6 mm) was exhibited for explants cultured on MS-medium containing 0.5mg/l BA. The highest number of shoots per explants(5.6) was obtained from callus grown on the medium supplemented with 2mg/l BA. These findings revealed that the use of high levels of BA enhanced organogenesis, however, reduction of shoot length occurred. This is in agreement with some earlier studies to assess the role of growth regulators on shoot initiation in *Ducrosia flabellifolia* [11], *Khaya grandifoliola* [14] and *Gypsophila paniculata* [4].

Table 4: Analysis of variance for the effect of NAA and BA and their interaction on, number of shoots per explants and shoot length (mm).

Source of variation	Mean of squares			
	df	Number of shoots per explants	Height of shoots (mm)	
BA	2	19.05**	219.36**	
NAA	1	$0.89^{n.s}$	12.17**	
BA×NAA	2	$1.05^{n.s}$	0.76**	
Error	12	0.44	0.05	

**: Highly Significant at 0.01 probability level, *: Significant at 0.05 probability level and n.s: Not significant.

Table 5: Means of Number of shoots per explants and Height of shoots (mm) as affected by BA and NAA.

Factor level			
NAA(mg/l)	BA (mg/l)	Number of shoots per explants	Height of shoots(mm)
	0.5	1.33 c	21.6 a
0	1	4 b	18.27 b
	2	5.67 a	10.27 c
	0.5	1.67 c	20.77 a
0.5	1	3.67 b	16.10 b
	2	4.33 a	8.33 c

*Means followed by the same letter(s) are not significant, different at 0.05 level of probability.

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