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Original Research

Cell dedifferentiation and multiplication of Burdock (*Arctium Lappa*) as a medicinal plant

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Abstract: *Arctium lappa* L. (Burdock) is an important plant with various pharmacological effects. According to the importance of this plant, optimization of its tissue culture will lead to more investigation and application of it. The aim of this study was to develop protocols for callus induction and shoot regeneration of *A. lappa*. In order to optimize of tissue culture in *A. lappa*, callus induction, indirect regeneration and direct regeneration were carried out in factorial experiment based on Completely Randomized Designs (CRDs). Hypocotyl and cotyledon were cultured on the Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of 2,4-Dichlorophenoxyacetic acid (2,4-D) and 6-Benzylaminopurine (BAP) for callus induction. In indirect regeneration experiment various levels of BAP and α-Naphthaleneacetic acid (NAA) and two types of explants (calli derived from cotyledon and hypocotyl) were investigated. In direct regeneration section, various levels of BAP plus 2 mg/l NAA and different explants (cotyledon, hypocotyl and bud) were compared. In both cotyledon and hypocotyl, the maximum callus induction was observed on a media containing 2 mg/l 2,4-D plus 1 mg/l BAP (100% and 76.19% respectively). The highest percentage of indirect regeneration (65%) was observed at 1 mg/l BAP plus 0.5 mg/l NAA on calli from hypocotyl. The highest percentage of direct regeneration (90.33) was observed in hypocotyl with a lateral bud explant on MS medium supplemented with 0.5 mg/l BAP plus 2 mg/l NAA. In this study, optimization of tissue culture protocol for *A. lappa* was carried out as a research technique, as well as technique for further exploitation of this plant.

Key words: Cell dedifferentiation; Callus induction; Plant regeneration; Burdock; Micropropagation.

Introduction

Arctium lappa L. or Burdock (Asteraceae) is a biennial plant native to Eurasia (1). Burdock contains various nutrients such as inulin, polyphenols, chlorogenic acid, proteins, carbohydrates, vitamins, amino acids, minerals, and unsaturated fatty acids (2,3). Additionally, this plant also has medicinal applications. A fraction has been isolated from the root has antitussive activity (4). Antioxidant, antimicrobial, anti-inflammatory, hypolipidemic and hepatoprotective effects of this plant have been reported (5-10). Burdock by inhibitory activity of α-glucosidase can be useful in diabetes, obesity and viral infections treating (11). It also contains inulin which has prebiotic properties (2). Compounds with anti-prostate cancer activity from *A. lappa* seeds were isolated (12).

Plant tissue culture is the best technique to exploit the cellular totipotency of plant cells for numerous practical applications and offers technologies for crop improvement (13). According to nutritional and medicinal properties of *A. lappa*, its propagation through tissue culture can be an alternative method for faster access to these products. In addition, seed germination rate and percentage are low due to dormancy. Hence, we can overcome this problem by using tissue culture technique. Also, optimization of tissue culture is an introduction to genetic engineering studies and further researches. Up to now a few researches have been reported on tissue culture of *A. lappa* (14). Therefore, the aim of current study was to develop a protocol for *in vitro* propagation of *A. lappa* from different explants.

Materials and Methods

Seed germination

The seeds were surface-disinfected by 70% (v/v) ethanol for 30 seconds followed by 2% (v/v) sodium hypochlorite (NaOCl) for 10 min and rinsed six times with sterile distilled water. In order to overcome seed dormancy, the seeds were placed between two layers of moistened filter paper (with 5 ml sterile distilled water) in sterilized petri dishes. The dishes were wrapped with parafilm and covered with aluminum foil. Then, they were stored for 21 days at 4°C. After overcoming of seed dormancy, the seeds were germinated in sterile culture bottles each containing 50 ml of DDW solidified with 0.8% (w/v) agar and maintained at 25±1°C under the 16 h light/ 8 h dark photoperiod. 7 days old seedlings were used for tissue culture.

Callus induction and shoot differentiation

MS basal medium (15) supplemented with 8 g/l agar and 30 g/l sucrose was used for tissue culture. Cotyledons and hypocotyls (explants) were placed on MS medium supplemented with 0, 1, 2, and 4 mg/l 2,4-D in combination with 0, 0.5, 1, 1.5 and 2 mg/l BAP (6-benzylaminopurine). The percentage of explants producing calli (CP) and percentage of necrotic explants (NP) were recorded after four weeks.

Induced calli from cotyledon and hypocotyl were

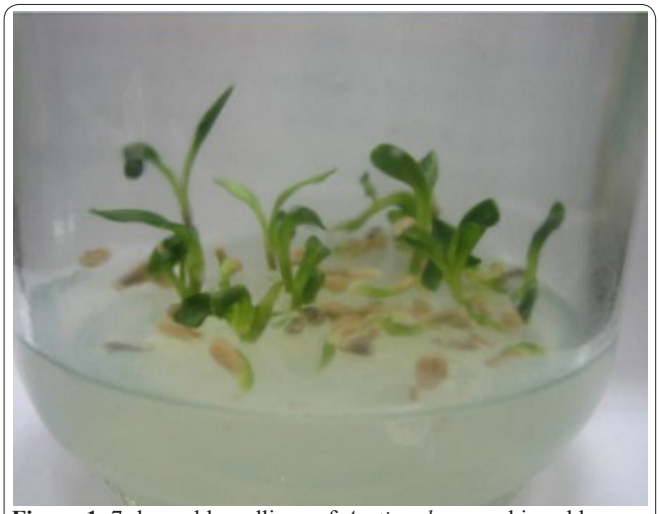

Figure 1. 7-days old seedlings of *Arctium lappa* achieved by germination of seeds on solidified DDW with 0.8% (w/v) agar.

transferred to MS medium supplemented with 0, 0.5 and 1 mg/l NAA in combination with 0, 0.5, 1, 2 and 3 mg/l BAP for shoot regeneration. The regeneration percentage and number of shoots initiated per explant were recorded.

Direct regeneration

For direct shoot regeneration, explants from cotyledon, hypocotyl and bud were cultured on MS medium supplemented with various concentrations of BAP (0.5, 1, 1.5 and 2 mg/l) plus 2 mg/l NAA. The regeneration percentage was recorded after four weeks. The pH of all media adjusted to 5.7 before autoclaving at 121°C for 20 min. The cultures were incubated in the growth room at 25±1°C under cool-white light with a 16 h photoperiod (40 to 60 µmol/m2/s) and subcultured on fresh media at 14 days' interval. Regenerated shoots were transferred to hormone-free MS medium for elongation. The elongated shoots were then transferred to MS medium supplemented with 1 mg/l IBA for rooting. For acclimatization, plantlets were transferred to pots filled with field soil, sand and perlite (1:1:1) and kept at 25±1°C and 70% relative humidity with a 16 h photoperiod in a growth chamber for 2 weeks.

Statistical analysis

All experiments were laid out as a completely randomized design in a factorial arrangement with three replications and each replication was made by using 3 petri dishes per medium. Analysis of variance was calculated and variations among treatment means were analyzed by Duncan's multiple range test (16) at P=0.05.

Results

Most seeds were germinated after 7 days of culture on solidified DDW with agar, while no germination occurred on MS basal medium (Fig. 1).

After 2 weeks of culture, callus (dedifferentiated cells mass) was initiated on the cut surfaces of explants (Fig. 2a and b). According to results of analysis of variance (Table 1), there were significant differences for callus induction among concentrations and combinations of 2,4-D and BAP (P<0.01). The highest frequency of callus formation (79.36% and 76.19%) and lowest percentage of necrotic explants (0%) was achieved on media supplemented with 0 mg/l BAP + 2 mg/L 2, 4-D and 1 mg/l BAP + 2 mg/l 2, 4-D respectively, for hypocotyl explants. While 100% callus formation and 0% necrotic explant was obtained on media supplemented with 2 mg/l BAP + 1 mg/l 2, 4-D and 0 mg/l BAP + 2 mg/l 2, 4-D and 1 mg/l BAP + 2 mg/l 2, 4-D for cotyledon explant (Table 2). The shoots were induced from the hypocotyl derived calli after 3-4 weeks, whereas most of the cotyledon derived calli were not regenerated (their data were ignored). The regeneration percentage and number of shoots per explant (callus) were depended on the concentrations and combinations of NAA and BAP (P<0.01, Table 3). Maximum shoot regeneration (65%) was obtained on MS medium supplemented with 0.5 mg/l NAA + 1 mg/l BAP, and the highest number of shoots per explant (4.78 and 4.33) were developed on media supplemented with 0.5 mg/l NAA + 1 mg/l BAP and 0.5 mg/l NAA + 2 mg/l BAP (Fig. 3, 4 and

Table 1. Analysis of variance for callus induction of *A. lappa.*

S.O.V	df	MS	
		Callus induction	Percentage of necrotic explants
Explant (A)	1	0.056**	0.165**
2,4-D (B)	3	3.704**	3.496**
AB	3	0.108**	0.022**
BAP (C)	4	0.326**	0.140**
AC	4	0.010**	0.046**
BC	12	0.409**	0.336**
ABC	12	0.042**	0.264**
Error	80	0.001	0.002
CV%		2.86	4.68

**: significant at 0.01 probability level, S. O.V: source of variations, df: degree of freedom, MS: mean square, CV: coefficient of variation.

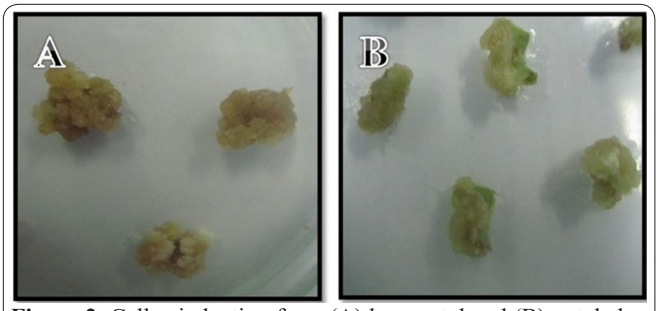

Figure 2. Callus induction from (A) hypocotyl and (B) cotyledon on MS medium containing 1 mg/l BAP + 2 mg/l 2, 4-D.

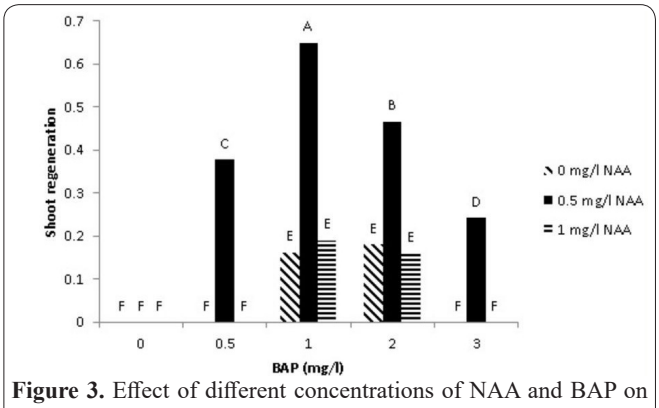

Figure 3. Effect of different concentrations of NAA and BAP on indirect shoot regeneration from hypocotyl derived calli of *A. lappa.*

Table 2. Effect of different concentrations of 2,4-D and BAP on callus induction from hypocotyl and Cotyledon explants and percentage of necrotic explants of *A. lappa* after 4 weeks of culture.

Growth regulator concentrations (mg/l)		Hypocotyl explant		Cotyledon explant	
2,4-D	BAP	CP	NP	CP	NP
0	0	0.00^{m}	14.29^{f}	0.00^{m}	57.14^{c}
0	0.5	0.00^{m}	0.00^{h}	0.00^{m}	0.00^{h}
0	1	0.00^{m}	0.00^{h}	0.00^{m}	0.00^{h}
0	1.5	0.00^{m}	0.00^{h}	0.00^{m}	0.00^{h}
0	2	0.00^{m}	0.00^{h}	0.00^{m}	0.00^{h}
1	0	52.38^{fg}	4.73^{g}	57.14^{ef}	42.86^{d}
1	0.5	14.29^{l}	0.00^{h}	38.10^{ij}	57.14^{c}
1	1	47.62^{gh}	0.00^{h}	95.24^{ab}	0.00^{h}
1	1.5	47.62^{gh}	33.33^{e}	85.71^{bc}	0.00^{h}
1	2	61.90^{e}	0.00^{h}	100^{a}	0.00^{h}
2	0	79.36^{cd}	0.00^{h}	100^{a}	0.00^{h}
2	0.5	85.71^{bc}	14.29^{f}	71.43^{d}	0.00^{h}
2	1	76.19^{cd}	0.00^{h}	100^{a}	0.00^{h}
2	1.5	14.29^{l}	0.00^{h}	0.00^{m}	59.05^{c}
2	2	33.33^{jk}	52.38^{cd}	14.29^{l}	14.29^{f}
4	0	42.86^{hi}	57.14^{c}	28.57^{k}	52.38^{cd}
4	0.5	0.00^{m}	100^{a}	0.00^{m}	100^{a}
4	1	0.00^{m}	100^{a}	0.00^{m}	100^{a}
4	1.5	0.00^{m}	95.24^{a}	14.29^{l}	80.95^{b}
4	2	54.76^{efg}	15.08^{f}	57.14^{ef}	71.43^{b}

Values within a column followed by different letters are significantly different at the 0.05 probability level, analyzed by Duncan's multiple range test.

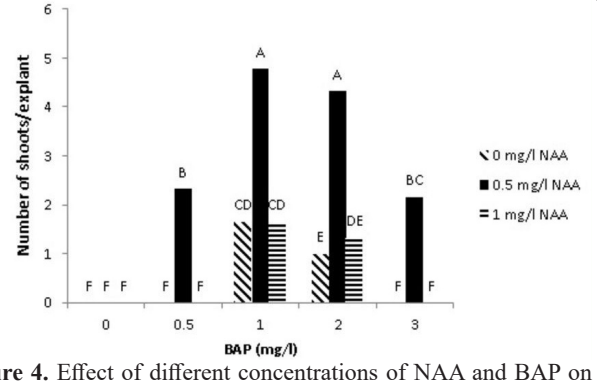

Figure 4. Effect of different concentrations of NAA and BAP on number of shoots regenerated per explant (callus) in *A. lappa*.

5a). Regenerated shoots were elongated in hormone-free MS medium for 2 weeks and then root induction were occurred on MS medium supplemented with 1 mg/l indol-3-butyric acid after 4-5 weeks (Fig. 5b, c and d). *In vitro* flowering was observed in some of plantlets (Fig. 5e and f).

The percentage of direct shoot regeneration was significantly affected by type of explant and concentrations of BAP ($P<0.01$, Table 4). Maximum shoot regeneration percentages (90.33% and 94.33%) were obtained on MS media supplemented with 2 mg/l NAA + 0.5 mg/l BAP and 2 mg/l NAA + 1 mg/l BAP in bud explant. The highest shoot regeneration percentage for cotyledon explant (69.67%) was observed on MS medium containing 2 mg/l NAA + 0.5 mg/l BAP and for hypocotyl explant (58%) was obtained on MS medium

Table 3. Analysis of variance for indirect shoot regeneration of *A. lappa*

S.O.V	df	MS	
		Shoot regeneration	Number of shoots/ explant
NAA	2	0.619^{**}	4.833^{**}
BAP	4	0.492^{**}	4.038^{**}
NAA × BAP	8	0.055^{**}	0.379^{**}
Error	30	0.001	0.014
CV%		4.06	9.09

**: significant at 0.01 probability level, S. O.V: source of variations, df: degree of freedom, MS: mean square, CV: coefficient of variation.

Table 4. Analysis of variance for direct shoot regeneration of *A. lappa.*

S.O.V	df	MS
		Shoot regeneration
Explant	2	0.456^{**}
BAP	3	0.261^{**}
Explant × BAP	6	0.043^{**}
Error	24	0.005
CV%		13.64

**: significant at 0.01 probability level, S. O.V: source of variations, df: degree of freedom, MS: mean square, CV: coefficient of variation.

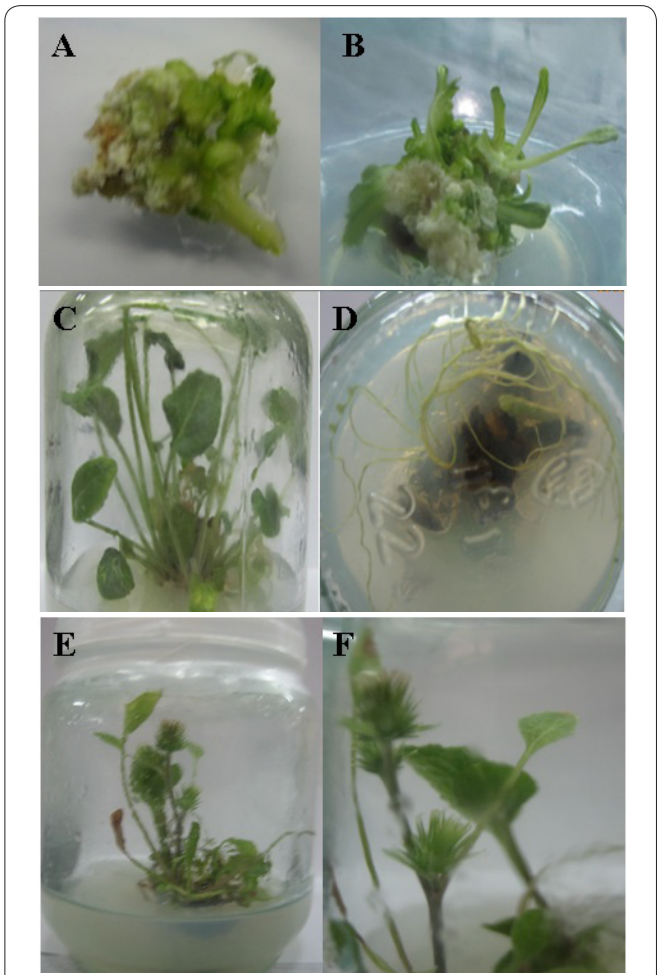

Figure 5. Different stages of indirect organogenesis in *A. lappa*, (A) indirect shoot regeneration from hypocotyl derived callus on MS medium supplemented with 0.5 mg/l NAA + 1 mg/l BAP after 3 weeks, (B) shoot elongation and multiplication on hormone-free MS medium, (C) and (D) transferring of regenerated shoots to MS medium supplemented with 1 mg/l IBA for rooting, (E) and (F) *in vitro* flowering.

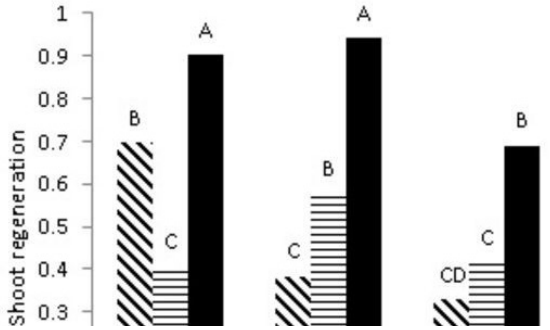

Figure 6. Effect of different concentrations of BAP on shoot regeneration from hypocotyl, cotyledon and bud of *A. lappa.*

containing 2 mg/l NAA + 1 mg/l BAP (Fig. 6).

Discussion

In general, the stimulation of tissue growth to form adventitious roots and adventitious buds depends on the relative ratios of auxin to cytokinin in the culture medium (17).

In our study, effect of an auxin (2,4-D) in combination with a cytokinin (BAP) can promote callus induction and shoot regeneration (Table 2, Fig. 3 and 6). This agrees with results for other plant species (18-26).

He *et al.,* (2006) describes a practice for plant regeneration from cultured seedling explants of *A. lappa* hypocotyls and cotyledons which were induced to form callus. The regenerated plants acclimatized in soil were normal morphologically and in growth characters. They flowered and set seed in the following year after acclimatization (14).

The highest frequency of shoot formation from hypocotyl (77.3%) and cotyledon (39.4%) has been reported in media containing 1 mg/l NAA + 1 mg/l BA and 2 mg/l NAA + 1 mg/l BA respectively (14).

He *et al.* (2006) reported that, the highest number of shoots per callus in *A. lappa* was obtained on MS medium supplemented with 2 mg/l NAA + 1 mg/l BA (14).

In this experiment it was found that there were significant differences among various explants of *Arctium. lappa* for callus induction and direct regeneration. The current study has resulted in the establishment of protocol for direct and indirect regeneration of *A. lappa* through hypocotyl, cotyledon and lateral bud explants. In this study, lateral bud explant of *A. lappa* showed a great potential for direct shoot regeneration.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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