Asian Journal of Chemistry Vol. 22, No. 3 (2010), 2383-2386

Effect of 6-Benzylaminopurine, 2,4-Dichlorophenoxyacetic Acid and Indole-3-Butyric Acid on Micropropagation Stages of *Achillea biebersteinii*

DANIAL KAHRIZI* and MEHDI KAKAEI†
Department of Biotechnology for Drought Resistance, Razi University, Kermanshah, Iran
E-mail: dkahrizi@yahoo.com

The yarrow (*Achillea biebersteinii*) is an important medicinal plant. Due to the limited plant material of this species, its micropropagation has been optimized in several stages. At first seeds placed on Murashig and Skoog medium in three different proportions (½, ¼ and complete Murashig and Skoog) for seed germination and seedling growth that ¼ Murashig and Skoog and Murashig and Skoog were best, for above, respectively. In direct shoot regeneration stage, the leaves have been placed on Murashig and Skoog medium supplemented with 6-benzylaminopurine (0, 2, 5 and 10 mg/L) that wasn't a significant difference for shoots regeneration and length average among 2, 5 and 10 mg/L 6-benzylaminopurine. For callus induction, there wasn't significant difference among 2, 4-dichlorophenoxyacetic acid concentrations (1, 2 and 5 mg/L) and 5 mg/L 6-benzylaminopurine was the best treatment for indirect regeneration. After elongation, in root induction stage, the young seedlings have been transferred to Murashig and Skoog medium supplemented with 0, 1, 2 and 4 mg/L indole-3-butyric acid that the 2 mg/L indole-3-butyric acid had the best effect on root formation.

Key Words: Micropropagation, *Achillea biebersteinii*, 6-Benzylaminopurine, 2,4-Dichlorophenoxyacetic acid, Indole-3-butyric acid, Regeneration.

INTRODUCTION

Because of increasing the poison of synthetic drugs, efforts have been directed to use medicinal plants[1,2]. Plant tissue culture refers to growing material plant on sterilized conditions. Plant tissue culture technique increases the quality and the rate of production[3].

Yarrow (*Achillea biebersteinii*) is a perennial herbaceous medicinal plant that grows widely in some regions of Iran and Europe and has many useful medicinal properties, such as diaphoretic, antihemorrhagic and antiinflammatory effects. Its essential oil is used to cure the nervous and rheumatic pains[4]. In one research, callus induction from leaf and stem explants of this plant on Murashig and Skoog

†Department of Plant Breeding, Young Researchers Club, Islamic Azad University, Kermanshah branch, Kermanshah, Iran.

medium supplemented with 2,4-dichlorophenoxyacetic acid and kinetin has been studied[5]. The effects of chloroform and aqueous extract of this plant on gram positive and gram negative bacteria and its extract effect on *H. pylori*, has been studied[6]. Due to the limited plant material of this species, efforts have been directed to micro-propagate this medicinal plant through *in vitro* culture with an *in vivo* to producing secondary metabolites.

EXPERIMENTAL

Aerial parts of *Achillea* were collected in some regions of west of Iran. Then these seeds were sterilized in sodium hypochlorite (1.5 %) for 5 to 10 min and so were placed on different proportions of Murashig and Skoog medium *i.e.*, Murashig and Skoog, ½ Murashig and Skoog, ¼ Murashig and Skoog, (with 20 g/L sucrose and 0.7 % agar). In second stage (after 10 days) young seedling leave were transferred to Murashig and Skoog medium supplemented with different concentrations of 6-benzylaminopurine (0, 2, 5 and 10 mg/L) and 0.1 mg/L 2,4-dichlorophenoxyacetic acid in order to direct shoot induction and study of its effects on direct regeneration. In this stage the effect of different concentrations of 2,4-dichlorophenoxyacetic acid (0, 1, 2 and 5 mg/L) with 0.1 mg/L kinetin on callus induction and Relative Growth Rate Callus (RGRC) and effect of above 6-benzylaminopurine treatments on indirect regeneration was investigated. In the shoot elongation stage, seedlings were placed on free plant growth regulator Murashig and Skoog medium. Then for root formation, the seedlings were transferred to Murashig and Skoog medium supplemented with 0, 1, 2 and 4 mg/L indole-3-butyric acid. After one week two parameters root induction per cent and root length were recorded. In all experimental designs, Completely Randomized Design (CRD) with four replications was used for statistical analysis. The Arc sin $\sqrt{x}$ data transformation was used for normalization. Analyses of variance (ANOVA) were carried out in order determine the effect of above mentioned treatments and the Duncan's multiple range tests was used to compare the mean performance.

RESULTS AND DISCUSSION

At first stage, statistical analysis of data showed that there is a significant difference among Murashig and Skoog medium for seed germination and seedling growth. The best seed germination and seedling growth was on the ¼ Murashig and Skoog and Murashig and Skoog medium, respectively (Table-1). This result may be related to poisonous and osmosis's effects of micro and macro elements on this plant, which it can reduce germination but they are necessary for seedling growth and establishment. The rate of Murashig and Skoog and ½ Murashig and Skoog medium salts may prevent the activation of some enzymes that related to germination[7,8]. But in ¼ Murashig and Skoog medium related enzymes have well functions. Other concentrations of Murashig and Skoog medium should be examined in order to reorganization of best concentration on germination in this wild type medicinal plant.

TABLE-1
MEAN PERFORMANCE OF DIFFERENT PROPORTIONS OF MURASHIG AND SKOOG (MS) FOR SEED GERMINATION AND SEEDLING GROWTH OF *Achillea Biebersteinii*

Proportions of MS	Seed germination (%)	Seedling growth (mm)
MS	79.25[b]	40.75[a]
½ MS	82.75[b]	33.50[b]
¼ MS	96.50[a]	25.50[c]

In each columns, data followed by the same letter are not significantly different.

In the second stage (SIM) in mentioned concentrations of 6-benzylaminopurine, number of shoots and shoot length average characters analyzed. Statistical analysis at these concentrations shows a significant difference among all 6-benzylaminopurine concentrations, but didn't show a significant difference among 2, 5 and 10 mg/L 6-benzylaminopurine (Table-2). This result may be related to endogenous and normal cytokinin rates. If the high level of endogenous cytokinin in plant is high, the rate of shoot induction will not be affected and the high levels of this plant growth regulator may have reverse effects on shoot induction[3]. In this stage shoot proliferation from all of 6-benzylaminopurine applied concentration were observed. This proliferation was due to direct regeneration.

TABLE-2
MEAN PERFORMANCE OF DIFFERENT CONCENTRATION OF 6-BENZYLAMINOPURINE (BAP) FOR DIRECT AND INDIRECT REGENERATION PARAMETERS OF *Achillea Biebersteinii*

BAP concentrations (mg/L)	SDR (%)	SLA (mm)	SIR (%)
0	5.00[b]	4.00[b]	12.75[c]
2	33.00[a]	19.75[a]	52.25[b]
5	31.00[a]	20.75[a]	81.75[a]
10	31.50[a]	23.75[a]	55.00[b]

In each columns, data followed by the same letter are not significantly different.

Statistical analysis showed that there was a significant difference among all different concentration of 2,4-dichlorophenoxyacetic acid for callus induction, but no significant differences among 1, 2 and 5 mg/L 2,4-dichlorophenoxyacetic acid (Table-3) for callus induction. The callus induction was showed in all 2,4-dichlorophenoxyacetic acid concentrations. The result of this research showed that *Achillea* has a high potential in callus induction.

Statistical analysis for root formation stage showed a significant difference among different concentration of indole-3-butyric acid for root induction and root length average (Table-4). Mean performance of different concentration showed that the 2 mg/L indole-3-butyric acid had the best effect on root induction and length average. Concentration of indole-3-butyric acid in more than 2 mg/L showed a negative effect on root formation parameters.

TABLE-3
MEAN PERFORMANCE OF DIFFERENT CONCENTRATION OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ON CALLUS INDUCTION OF *Achillea biebersteinii*

2,4-D concentrations (mg/L)	Callus induction (%)	RGRC (%)
0	2.750[b]	5.08[b]
1	24.50[a]	26.37[a]
2	25.75[a]	29.46[a]
5	27.25[a]	24.75[a]

In each columns, data followed by the same letter are not significantly different.

TABLE-4
MEAN PERFORMANCE OF DIFFERENT CONCENTRATION OF INDOLE-3-BUTYRIC ACID (IBA) ON ROOT FORMATION PARAMETERS OF *Achillea biebersteinii*

IBA concentration (mg/L)	Root induction (%)	Root length average (mm)
0	69.75[b]	3.56[b]
1	73.25[b]	4.08[b]
2	89.25 [a]	8.48[a]
4	69.25[b]	3.05[b]

In each columns, data followed by the same letter are not significantly different.

Conclusion

The best seed germination and seedling growth was on the ¼ Murashig and Skoog and Murashig and Skoog medium, respectively. In the shoot induction phase in mentioned concentrations of 6-benzylaminopurine, number of shoots and shoot length average didn't show a significant difference among 2, 5 and 10 mg/L 6-benzyl-aminopurine. There were no significant differences among 1, 2 and 5 mg/L 2,4-dichlorophenoxyacetic acid for callus induction. In root formation stage showed a significant difference among different concentration of indole-3-butyric acid for root induction and root length average

REFERENCES

1. A. Moieni and D. Kahrizi, Plant Tissue Culture Practice, Tehran Basij Press (2003).
2. In http://www.imp.ac.ir/publications/journal/Journal7/Abstract.pdf.
3. D. Kahrizi, A. Arminian and A. Masumi Asl, *In vitro* Plant Breeding, Razi University Press (2007).
4. B. Ozlem, G. Medine, S. Fikrettin, O. Hakan, K. Hamdullah, O. Hakan, S. Munevver and O. Tulin, *Turk. J. Biol.*, **30**, 65 (2006).
5. A. Jaime, S. Teixeira and M. Anthemideae, *Afr. J. Biotechnol.*, **2**, 547 (2003).
6. M. Satish, A. Nalawade, P. Sagare, Y. Chen, C. Lee, K. Lin and T. Hsin-Sheng, Studies on Tissue Culture of Chinese Medicinal Plant Resources in Taiwan and their Sustainable Utilization, Taiwan University Thesis (2002).
7. S. Kirmizi and G. Guleryuz, *Asian J. Plant Sci.*, **6**, 374 (2007).
8. W.B. Wang, Y.H. Kim, H.S. Lee, K.Y. Kim, X.P. Deng and S.S. Kwak, *Plant Physio. Biochem.*, **47**, 570 (2009).

(*Received*: 8 August 2009; *Accepted*: 3 December 2009) AJC-8134