Effects of Various Gelling Agents on Growth and Development of Adventitious Buds of Purple Coneflower (*Echinacea purpurea*)

N Dahanayake[1*] and YY Sheng
[1*]Department of Agricultural Bioogy, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya
[2]South China Agricultural University, Guangzhou, China

Abstract

Purple coneflower is a perennial medicinal herbaceous plant belongs to family Asteraceae, native to Midwestern and Southeastern United States. In addition to medicinal value, it has received considerable attention in recent years for its value in landscape and in florist bouquets. Gelling agents are used in plant tissue culture to solidify the nutrient medium. In this study, we examined the growth and development of purple coneflower in MS basal medium incorporated with different gelling agents. Petiole explants were grown on the MS basal medium with 0.3 mg/l BA and 0.01 mg/l NAA as sole plant growth regulators with different concentrations of gelling agents (agar 0.5%, phytagel 0.2%, cold-gel gum 0.7%, carrageenan 0.6%) and weight of whole plant (g), weight of leaf (g), weight of petiole (g), weight of root (g) and weight of residues (g) were measured separately from the regenerated plants Significantly high *weight of whole plant (*1.56 g) was observed in the medium with agar while the least *weight of whole plant (*0.4172 g) was observed in the medium which was solidified by Carrageenan. Among the tested gelling agents, 0.5% agar and 0.2% phytagel were found more effective than cold-gel gum and carrageenan for improving shoot growth and development of the plant.

Key words: Purple coneflower, Gelling agents, Tissue culture, MS basal medium

Introduction

Echinacea is one of the most important medicinal herbs in commerce today (Foster 1985), and has a long history of medicinal use dating back to the Plains Indian tribes of mid-Western merica (Foster 1990; Kindscher 1989; Shemluck 1982). *E. purpurea* has been used extensively in medicinal preparations for the treatments of many diseases, including colds, upper respiratory infections, toothache, headache, stomach cramps, snake bites, rabies and wound infections (Bauer 1999). The purple coneflower is often grown simply for its ornamental value, especially for its showy flowers.

Time has come to increase the production of *Echinacea purpurea* in terms of quality and quantity, to achieve its potential yield in order to meet the global increasing demand. Plant biotechnology can be used to open new possibilities for improving *E. purpurea* with such characteristics in order to fulfill the increasing global demand (Murch et al. 2000). Agar is the most frequently used solidifier in plant tissue culture media (Afrasiab and Jafar, 2011) and also the most expensive component in the medium. Various brands and grades of agar, agarose, phytagel and gelrite were used for in vitro micro-propagation (Debergh 1983). More than 100 years ago, agar has been widely used as a gelling agent in plant tissue culture technique. This is because its stability, high clarity, non toxic nature and resistance to its metabolism (Henderson and Kinnersley 1988). Some studies have been made to find alternative substances that have same ability as agar and also effect of using agar substitutes in order to reduce cost in preparing tissue culture media Ex:- Cassava powder. The successful application of in vitro methods is greatly dependent on a reliable regeneration system. Effects of different gelling agents were studied to develop and optimize- the protocol to achieve higher efficiencies on the growth and development of Purple cornflower through micro-propagation, in the current study.

Materials and Methods

Plant source

Seeds of purple coneflower were purchased from the supermarket (Distributer: Plantation products, Norton, MA 02766, USA) and cultivated at the Chinese Medicinal Plant Garden in the campus of South China Agricultural University. Seeds were collected from these seed-grown plants and used in the present study.

Establishment of aseptic seedlings

Seeds were surface-sterilized by immersing in 70% ethanol for 1 minutes and soaking in a 0.1% mercuric chloride for 10 minutes followed by 1% sodium hypochlorite solution containing one drop of Tween 20 per 50 ml for 10 minutes. Sterilized seeds were then rinsed three times in sterile deionized water and inoculated on a medium comprised of half-strength MS (Murashige and Skoog 1962) salts, 1% sucrose and 500mg/l lactalbumin hydrolysis and the medium was solidified with 0.2% phytagel prior to autoclaving. After 14 days under dim-light, germinated seeds were transferred to a medium containing full-strength MS salts and 1% sucrose, and 0.2% phytagel and the pH was adjusted to 6.0. Cultures were then incubated in a room of 25-27 ^{0}C and 12-h photoperiod under cool-white light (50µmol/m^2/s) (Toshiba, Japan).

All the media used were adjusted to the pH 6.0 with 1N NaOH or 1N HCl solution, gelled with 0.6% agar prior to autoclaving at 1.4kg/cm^2 for 20 minutes. All treated explants were kept in lighted conditions with a 12h photoperiod under cool-white light (50µmol/m^2/s) (Toshiba, Japan) at 250C for 40 days.

Investigating the effects of different gelling agents in the culture system

Exposure of petiole explants to the MS basal medium with 0.3 mg/l BA and 0.01 mg/l NAA as the sole plant growth regulators with different gelling agents namely agar 0.5%, phytagel 0.2%, cold-gel gum 0.7% and carrageenan 0.6% were used to evaluate the growth and development of the plant.

Data collection and analysis

Cultures were evaluated 40 days after initiation. Regeneration percentage was calculated based on the ratio of number of explants regenerated buds to the number of explants cultured. The number of shoots produced was also recorded to evaluate the shoot formation in response to the different gelling agents. The whole plant was weighed to estimate the plant growth, and, 40 days after in bud growing cultures, vegetative part and roots were also weighed, separately. All experiments reported here were repeated at least once with a minimum of four replicates, each with eight explants per bottle. Statistical analysis was carried out using the Student Newman-Kuells Means Separation Test of SAS (SAS Institute, Cary, NC 1995).

Results and discussion

Results of the studies were analyzed and the effects of treatments in each experiment were compared on organogenic regeneration. In addition to quantitative data, the qualitative responses were also recorded in the study. Significantly highest weight of whole plant (1.56 g) was observed in the medium with agar while least weight of whole plant (0.4 g) was observed in the medium which was solidified by Carrageenan. Among the tested gelling agents, 0.5% agar and 0.2% phytagel were found more effective than cold-gel gum and carrageenan for improving shoot growth and development of the plant (Table 1).

MS medium proved a satisfactory basic medium for micro-propagation of E. purpurea and protocols were established for initiation, proliferation, rooting and weaning. In addition to the problems associated with establishing sterile seedlings, E. purpurea was found to have different responses to different plant growth regulators.

Table 1. Comparison of the effects of various gelling agent on growth of adventitious buds

Gelling agent	Mean Weight of whole plant (g)	Mean Weight of leaf (g)	Mean Weight of petiole (g)	Mean Weight of root (g)	Mean Weight of residues (g)
Agar	1.56^{a*}	0.55^{a}	0.40^{a}	0.47^{a}	0.09^{a}
Phytagel	0.85^{b}	0.31^{b}	0.26^{b}	0.31^{b}	0.08^{a}
Cold gel gum	0.59^{bc}	0.23^{bc}	0.13^{c}	0.14^{c}	0.04^{a}
Carrageenan	0.41^{c}	0.16^{c}	0.12^{c}	0.07^{c}	0.04^{a}

*** In each column, means fallowed by the same lower case letter(s) are not significantly different at 5% level in Duncan's Multiple Range Test. DMRT**

Agar is being the most commonly used gelling agent to solidify the media in plant tissues culture experiments. However, there are doubts about the inhibitory effect of raw agar on the growth and development of explant. Keeping in view this idea, we conducted a comparison among agar, phytagel, cold-gel gum and carrageenan. Gelling agents known to have comparatively more favorable influence on explant growth in the culture medium. There was no a remarkable difference between 0.5% agar and 0.2% phytagel and it was found more effective than cold-gel gum and carrageenan on regeneration of explants.

Conclusions

For the petiole segment of Echinacea purpurea, 0.5% agar and 0.2% phytagel were found more effective than cold-gel gum and carrageenan on regeneration of explants.

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