Induction of Polyploids from Purple Coneflower (*Echinacea Purpurea* L.) through Colchicine Treatments

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Abstract

In this study petiole explants were obtained from *in vitro* grown diploid *E. purpurea* (Purple coneflower: Asteraceae) plantlets and shoots were regenerated by culturing the explants on MS basal medium containing 0.3 mg/l BA, 0.01 mg/l NAA and four different concentrations (30, 60, 120 and 240 mg/l) of colchicine for 30 days or 120 mg/l of colchicine for various durations (7, 14, 21, and 28 days). The regenerated shoots were induced to root on MS basal medium with 0.01 mg/l NAA, then the root-tips of the regenerated shoots were sampled and the chromosome number of the root tip cells was counted. Completely Randomized Design (CRD) was used with 5 replicates. It was found that a treating duration longer than seven days was necessary for the induction of tetraploid shoots, and treating the explants with 120 mg/l colchicine for 28 days was the most efficient conditions for the induction, yielding 23.5% tetraploid among all the regenerated shoots. These newly established protocols in organogenesis, doubling the chromosome number of diploid *E. purpurea* plants have high applicable values for genetic improvements of the crop.

Key words: Purple coneflower, Colchicines, Diploid, Tetraploid

Introduction

The importance of polyploid plants in agriculture is well documented (Lewis, 1980). Nevertheless, only few cases of polyploid medicinal plant have been reported (Gao, 1996). Inducing polyploid plants by tissue culture is advantageous because of high affectivity and convenience in comparison with traditional methods (Gao *et al.*, 1996).

The manipulation of ploidy is a valuable tool in improving crops and also very useful tool in plant breeding, that has been used to overcome hybridization barriers (dissolving interploid blocks), restore hybrid fertility by the creation of allopolyploids, develop sterile cultivars (meiosis being prevented by complications due to the presence of multiple homologues chromosomes), enhance pest resistance and disease tolerance in allopolyploids by the additive effect of defense chemicals inherited from both parents, and create larger plants with enhanced vigor (Stebbins, 1971). Polyploids usually show different morphology compared with diploids, the amount of differences being very dependent on the plant species, the degree of heterozygosity, the ploidy level, and the mechanism relating to gene silencing, gene interactions, gene dose effects and regulation of specific traits and processes (Leitch and Bennett, 1997). Out of many applicable methods, the use of chemicals to induce changes in chromosome number has been well established. Colchicine is the most frequently used and the most efficient agent to induce chromosome doubling in plants. Varying environmental conditions are also suspected of inducing the appearance of mixoploidy (Sikdar and Jolly, 1994).

Moreover, the treatment is lengthy, laborious, carcinogenic effect and uneconomical due to a greater consumption of colchicine during the treatment. Polyploids often generate variants that may contain useful characteristics. In addition, by doubling the gene products, polyploids provide a wider germplasm base breeding studies to obtain superior new varieties for the commercial production. For those benefits, we described via these experiments the main factors influencing the efficiency in doubling the chromosome

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number, colchicine concentration and treatment duration in the regeneration medium. The aim was to establish effective methods for doubling chromosome number and obtaining pure tetraploid plants with higher mass production from diploid *E. purpurea* plants.

Materials and methods

Induction of chromosome doubling

Comparison of the effects of the different concentrations of colchicine

Petiole explants (5-10 mm) excised from the sterile diploid *E. purpurea* were cultured on MS basal medium containing 0.3 mg/l BA with 0.01 mg/l NAA and different concentrations of filter sterilized colchicine (0.0, 30, 60, 120, 240, 480 mg/l) which was dissolved in 2% DMSO. Explants were pre-cultured on shoot regeneration medium for one week to heal the cutting wound and initiate cell division, and then transferred to shoot regeneration medium with colchicine. After 30 days interval colchicine treated petiole explants were transferred to the same medium without colchicine. 7-10 explants were cultured in one bottle.

Comparison of the effects of different duration of treating of colchicine

From the results of above experiment choose the best colchicine concentration to double the chromosome number and conduct the following experiments to investigate the effect of treatment duration to produce the polyploids.

Prepared 30 bottles of regeneration medium (0.3 mg/l BA + 0.01 mg/l NAA) supplemented with 120 mg/l colchicine and transferred the explants from day 7, 14, 21, 28 and 35 to fresh medium with the same composition without colchicine and cultured 40 days.

Root induction of regenerated plants

Healthy and robust regenerated shoots longer than 1.5 cm were cut from the mother tissues and cultured to MS medium with 0.01 mg/l NAA for the initiation of roots and further growth of the intact regenerated plantlets.

Observations of chromosomes and determination of ploidy level

Fifty regenerate plants were randomly selected actively growing root tips about 5-10 mm in length were excised. These root tips were pre-treated with 0.01% colchicine solution at room temperature for 2-3 hours. Then they were transferred to fixing in Carnoy's solution containing acetic acid: ethanol (95-100%) (1: 3, v/v) for at least 24 hours. Then root tips were hydrolyzed in 1 N HCl for 05-10 min at 65°C. Root tips were stained with one drop of carbol fuchsin solution for 1-2 minutes, squashed under cover glass and cell samples of the root tips were observed for chromosomes under a microscope (Leica DLMB2).

Data analysis

All experiments were arranged Complete Randomized Design (CRD) with 5 replicates. Statistical analysis was carried out using the Student Newman-Kuells Means Separation Test of SAS program (9.1.3).

Results and discussion

Increasing the chromosome number of the explants after colchicine treatments depended on the concentration and on the duration of the treatment.

Effects of the different concentrations of colchicine

The regeneration rates in the colchicine treatments were lower than those of the control, epecially at higher concentrations and longer durations. The first visible effect was shoot regeneration ability and growth of buds delayed significantly on colchicine containing medium compared with non treated regeneration medium (Fig. 1), especially higher concentrations and

longer durations inhibited more heavily. Shoot regeneration from petiole explants generally took place after one month of culture on regeneration medium, and the regenerated shoots could continue grow on the same medium for another 10 days without declining in activity. Colchicine also delayed the regeneration progress and induced noticeably more callus on the cutting surface of the explants. After a month of growth, all untreated explants had significantly higher number of shoots than colchicine treated explants where as colchicine treated explants had shorter shoots comparing to the control. The highest concentration (480 mg/l) and maximal treatment duration (35 days) prevented completely the regeneration of explants.

When treated with high colchicine concentration for 30 days, the percentages of explants that died increased. Non growing, brown buds were considered to be dead. The surviving calluses were subcultured to the MS basal medium after 30 days in order to develop buds and/or plantlets. Explants survived on the media with colchicine concentrations of 0.0, 30, 60, 120, 240 mg/l showed 97.5%, 67.5%, 50%, 20% and 7.5% regeneration respectively. However, all of the explants treated with 480 mg/l colchicine died.

30 mg/l was not effective because only a low portion cells had doubled chromosome numbers. 120 mg/l colchicine in the medium exhibited the best doubling effects; among 51 plantlets examined, chromosomes in 11 plantlets were confirmed to have been completely doubled. Colchicine at 60 and 240 mg/l was also effective, being able to induce complete chromosome doubling in 5.9 - 7.8% of the examined plantlets, but in comparison with 120 mg/l, these two concentrations were lower efficiency.

Effects of different duration of treating of colchicine

In the above experiments, 120 mg/l colchicine treatment induced chromosome doubling with the highest frequency. In this experiment, explants were inoculated on media supplemented with 120 mg/l colchicine for various durations. It was clear that longer duration treatments of colchicine inhibited the regeneration efficiency. No shoots survived from the too highest treatment duration (35 days) and the all explants died without showing regeneration. The surviving calluses were sub-cultured to the MS basal medium without colchicine after 7, 14, 21 and 28 days interval to develop buds and/or plantlets. All of the colchicine treated explants survived, showing 72.5%, 60%, 47% and 17.5% regeneration respectively. However with all explants which were treated 35 days had 100% lethality. When increasing the treatment

Figure 1. Cultures of petiole explants on regeneration medium containing 0.3 mg/l BA and 0.01 mg/l NAA a. for 40 days without subculture b. for 40 days after being cultured on a medium containing 0.3 mg/l BA, 0.01 mg/l NAA and 120 mg/l colchicine for 30 days

duration, the percentages of explants that survived were reduced simultaneously.

Roots were sampled from plantlets growing from the regenerated shoots and chromosome number of the root-tip cells was counted. The highest percentage (23.5%) of tetraploid induction occurred in the regeneration medium treated with 120 mg/l colchicine for 28 days. However, 14 and 21 days treated explants also generate a substantial number of tetraploids (11.7% and 19.6%) whereas no tetraploid observed in treatment for 7 days. Thus, this method provides an efficient technique by which colchicine can be utilized economically for the mass induction of tetraploids.

Petiole explants with 120 mg/l colchicine for 28 days was more effective for the induction of tetraploids in purple coneflower.

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