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EFFECTS OF PLANT GROWTH REGULATORS ON THE MORPHOGENESIS OF CAULIFLOWER CURD TRANSVERSE THIN CELL LAYER EXPLANTS

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SUMMARY

Transverse thin cell layers (tTCLs) from mature curd of Cauliflower (*Brassica oleracea* var. *botrytis*) were cultured on Murashige and Skoog medium supplemented with different types of plant growth regulators (PGRs) at various concentrations. The results were recorded after 6 weeks of culture. The presence of 2,4-dichlorophenoxy-acetic acid (2,4-D) at 0.1 - 1.0 mg l^{-1} resulted in callus formation, while roots formed in culture medium supplemented with 0.5 - 1.0 mg l^{-1} NAA (naphthalene acetic acid). When 1-phenyl-3-(1,2,3-thiadiazol-5-yl)-urea (TDZ) was added to culture medium at 0.2 - 0.6 mg l^{-1}, shoot clusters regenerated directly from the edge of explants. Shoots initiated in the presence of TDZ, but without stem elongation and leaf formation. In order to obtain normal plant development, clumps of regenerated shoots were transferred onto PGR-free MS medium. Approximately 26 shoots with normal stem elongation developed from each tTCL after 2 weeks. Rooting was obtained by transferring shoots to MS medium supplemented with 0.4 mg l^{-1} NAA and 0.2 mg l^{-1} Kinetin. Regenerated plants with adventitious roots were transferred to soil. The results demonstrated that the choice of PGRs is of significance in determining the morphogenesis of cauliflower curd tTCL explants.

Keywords: *Brassica oleracea var. botrytis, curd, TCL, morphogenesis, PGRs*

INTRODUCTION

Cauliflower is one of the most important vegetable crops in the world because of their nutritious value. According to the United States Department of Agriculture, 100 g of raw cauliflower provides 77% of an adult's Dietary Reference Intakes (DRI) of vitamin C. It is also a source of dietary fiber, vitamin B6, folate, pantothenic acid, as well as small amounts of other vitamins and minerals.

The low price of cauliflower seeds has restricted the use of clonal multiplication for breeding purposes on this important crop. Previous studies on *in vitro* propagation of cauliflower are limited to seedling explants (Vandemoortele *et al.*, 1993; Dash *et al.*, 1995; Arora *et al.*, 1996; 1997), protoplast culture (Delpierre, Boccon-Gibod, 1992; Yang *et al.*, 1994) and anther culture (Yang *et al.*, 1992). Other different explants from vegetative (including stem, petiole, leaf, leaf rib) and floral (including peduncle, pedicel, flower bud and curd) tissues of cauliflower were also used for *in vitro* propagation (Prem, Nicole, 1999).

Our previous research reported that "thin cell layer" explants from the surface of floral branches of tobacco could be induced to form either callus, vegetative buds, flowers or roots by adjusting the pH and the ratio of auxin to cytokinin in the culture medium (Nhut *et al.*, 2001). In this paper, we describe the morphogenesis of cauliflower curd transverse thin cell layers in culture medium supplemented with different types of PGRs.

MATERIALS AND METHODS

Plant materials

Mature curds (approximately 20 - 25 cm in diameter) were collected from field. These curds were sliced into small pieces. Each curd piece was washed thoroughly under running tap water for 30 min, soaked in detergent (Viso, Dongnai, Vietnam) for 5 min, rinsed 6 times with distilled water and then with ethanol (70%) for 30 s. After three rinses with distilled water, the small piece of curd were disinfected with 0.1 % $HgCl_2$ for 6 mins, and rinsed 6 times in sterile distilled water. These curds were cut into rounds (1 mm thickness transverse slices) (Figure 1).

Media and experimental conditions

TCLs were placed on MS medium (Murashige,

Skoog, 1962) containing 30 g l^{-1} sucrose, 8 g l^{-1} agar and 2,4-D (0.1, 0.5 or 1.0 mg l^{-1}), NAA (0.1, 0.5 or 1.0 mg l^{-1}) or TDZ (0.2, 0.4, 0.6, 0.8 or 1.0 mg l^{-1}).

In all experiments, culture media were dispensed into culture vessels (250 ml), each containing 30 ml medium and capped with a transparent polypropylene film. Culture media were adjusted to pH 5.8 before autoclaving at 121°C for 20 min. All cultures were incubated at 25 ± 1 °C with a photoperiod of 16^h per day at a light intensity of 40 µmol m^{-2} s^{-1} fluorescent light. Data were recorded after 45 days culture. The data was analyzed for significance by analysis of variance with mean separation by Duncan's multiple range test.

RESULTS AND DISCUSSION

In this study, a protocol was developed for controlling the type of morphogenesis that occurs in cauliflower mature curd explants when cultured on media with different types of PGRs. Each of PGRs stimulated distinct morphogenetic pathways. These PGRs were shown to stimulate the direct formation of tissues or organs such as shoots, roots or calli depending on the medium on which tTCLs were cultured (Figure 2).

tTCL explants of cauliflower curd in PGR-free MS medium enlarged significantly after 7 - 8 days culture. These explants, however, turned brown and became necrotic after 4 weeks culture. These results were also consistent with the report on the morphogenetic capacity of TCL explants of sugar beet, which was strongly dependent on the presence of PGRs in the medium (Detrez *et al.*, 1988).

Effect of 2,4-D on callus formation

Callus from tTCL explants of cauliflower curd showed enhanced growth on the medium supplemented with 2,4-D at different concentrations. tTCL explants cultured in medium supplemented with 1.0 mg l^{-1} 2,4-D produced callus with the highest frequency (Table 1). In the presence of 2,4-D at lower concentrations, few calli turned brown and necrosis. Browning callus percentages at the concentrations of 0.1 and 0.5 mg l^{-1} are 6.7% and 3.3%, respectively.

Callus formation may be due to the ratio of cytokinin to auxin as mentioned by Skoog and Miller (1957) and Gaspar *et al.* (2003). In the present research, primary callus was friable, globular and yellowish-white by utilizing different concentrations of 2,4-D (Table 1, Figure 2a). These calli subsequently gave rise to different kinds of callus when continuously proliferated in the same medium.

Effect of NAA on root formation

Experiments on different NAA concentrations revealed that high frequencies of root organogenesis occurred at 0.5 - 1 mg l^{-1} NAA (100%), but primary root number, primary and adventitious root length on medium supplemented with 0.5 mg l^{-1} NAA were higher than other media (Table 2).

Table 1. Effect of 2,4-D on callus formation of cauliflower curd tTCL explants.

2,4-D concentrations (mg l^{-1})	Callus formation rate (%)	Callus fresh weight (g)
0.1	93.3	0.97^b
0.5	96.7	1.25^a
1.0	100	1.02^b

* Different letters within a column indicate significant differences at $\alpha = 0.05$ by Duncan's multiple range test.

Table 2. Effect of NAA on root formation of cauliflower curd tTCL explants.

NAA concentrations (mg l^{-1})	Root formation rate (%)	Root length (mm)	Number of roots
0.1	94.4	15.2^b	15.3^b
0.5	100	21.8^a	16.7^a
1.0	100	18.3^b	15.0^b

* Different letters within a column indicate significant differences at $\alpha = 0.05$ by Duncan's multiple range test.

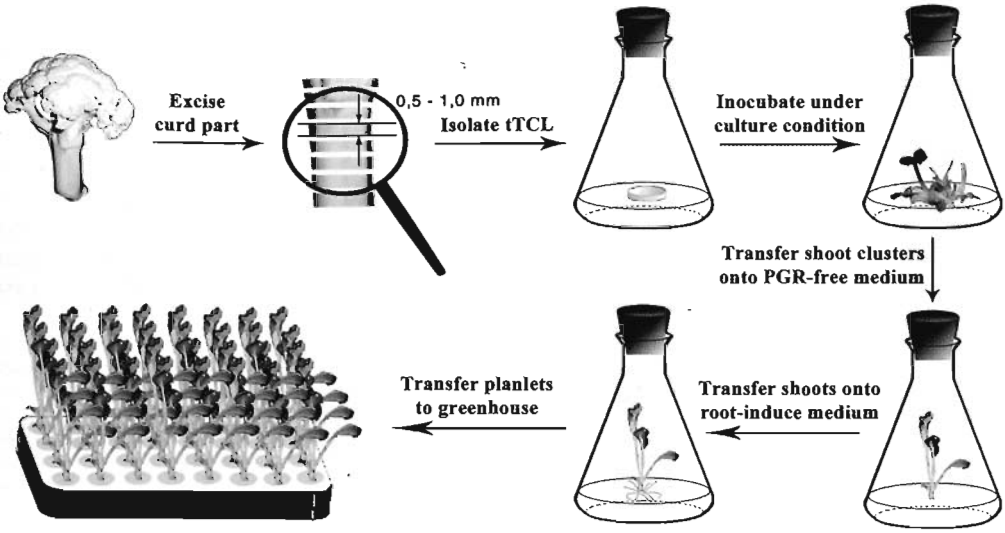

Figure 1. Diagram of cauliflower morphological pathway by using transverse thin cell layer technology.

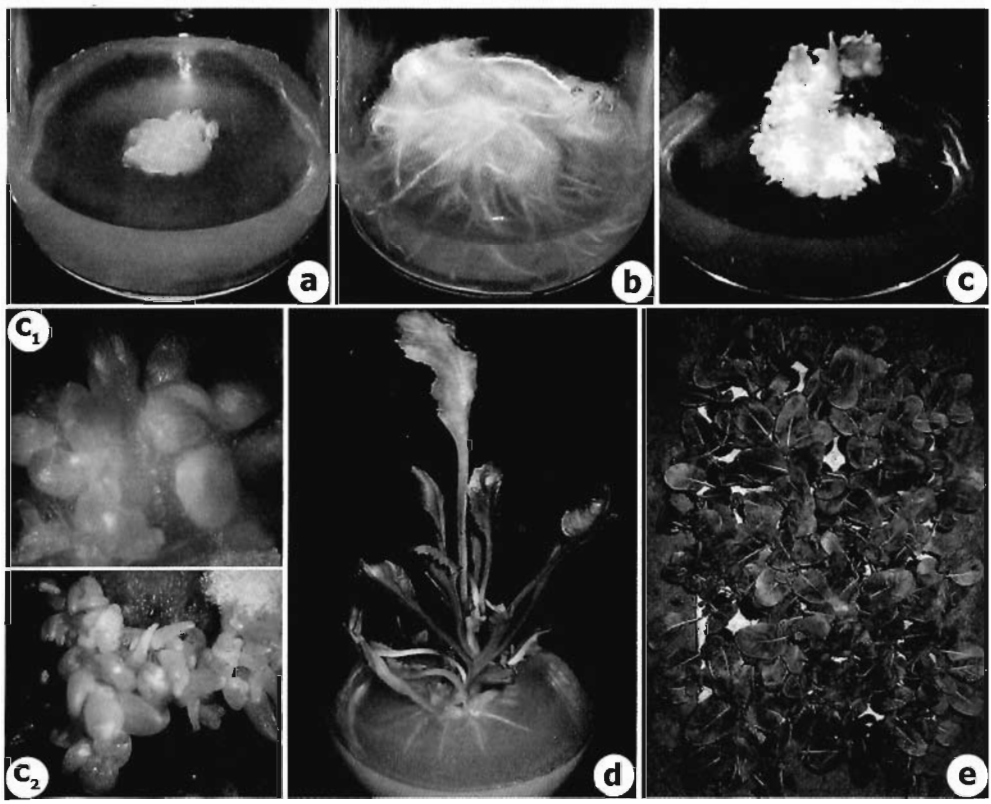

Figure 2. Callus (**a**), root (**b**), shoot (**c, c_1, c_2**), induction from curd tTCL of cauliflower; plantlet formation (**d**), and *ex vitro* performance (**e**).

Effect of TDZ on shoot formation

For TDZ, bud primordia were initiated on 100% tTCL explants with the concentrations ranging from 0.2 - 0.6 mg l^{-1}. An average of 26 bud primordia per tTCL was obtained at 0.6 mg l^{-1} of TDZ (Table 3).

At higher concentrations of TDZ, bud primordia formed but their further development was reduced. In order to obtain normal plant development, clumps of regenerated shoots were transferred onto PGR–free MS medium.

The effect of TDZ as cytokinin-like substances (Mok *et al.,* 1987), as well as their effect on shoot regeneration in *in vitro* cultures (Hosokawa *et al.,* 1996) were demonstrated. Other authors have also reported TDZ effect on organogenesis of peanut embryo sections and hypocotyl (Saxena *et al.,* 1992) and on *Geranium* seedlings (Gill *et al.,* 1993). In this research, we obtained high bud regenerative frequency by employing tTCL method combining with the ultilization of TDZ on *Brassica oleracea* var. *botrytis*.

Table 3. Effect of TDZ on shoot regeneration of cauliflower curd tTCL explants.

TDZ concentrations (mg l^{-1})	Shoot regeneration rate (%)	Fresh weight of shoot clusters (g)	Number of shoot per tTCL explant
0.2	100	2.28^{c}	22^{b}
0.4	100	2.53^{ab}	23^{b}
0.6	100	2.62^{a}	26^{a}
0.8	96.4	2.47^{b}	20^{c}
1.0	84.2	2.21^{c}	19^{c}

Different letters within a column indicate significant differences at $\alpha = 0.05$ by Duncan's multiple range test.

CONCLUSION

In this study, by using tTCLs (1 mm thickness) and various types of plant growth regulators, a simple and highly effective method for successfully programming morphogenesis for callus, root, and shoot formation was achieved. The addition of selected auxins and cytokinins to the culture medium and the size of explant improved the specificity of morphogenesis. It was demonstrated that tTCL was an explant source, which was very sensitive to the presence of plant growth regulators in the medium and this has not been observed when other cauliflower tissues or organs were used.

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ẢNH HƯỞNG CỦA CÁC CHẤT ĐIỀU HÒA SINH TRƯỞNG THỰC VẬT LÊN SỰ PHÁT SINH HÌNH THÁI CỦA MẪU CẤY LÁT MỎNG TẾ BÀO CUỐNG CHỒI HOA SÚP LƠ

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TÓM TẮT

Các mẫu cấy lát mỏng tế bào cắt ngang (tTCL) từ cuống chồi hoa của cây Súp lơ (*Brassica oleracea* var. *botrytis*) được nuôi cấy trên môi trường MS bổ sung các chất điều hòa sinh trưởng thực vật ở những nồng độ khác nhau. Kết quả được ghi nhận sau 6 tuần nuôi cấy. Sự hiện diện của 2,4-dichlorophenoxy-acetic acid (2,4-D) ở nồng độ 0,1 - 1,0 mg/l cảm ứng sự hình thành mô sẹo trong khi rễ được cảm ứng trên môi trường có bổ sung 0,5 - 1,0 mg/l NAA (naphthalene acetic acid). Khi 1-phenyl-3-(1,2,3-thiadiazol-5-yl)-urea (TDZ) được bổ sung vào môi trường nuôi cấy ở nồng độ 0,2 - 0,6 mg/l, các cụm chồi được hình thành từ rìa của mẫu cấy. Tuy nhiên, chồi được tạo thành trên môi trường có TDZ không có sự phát triển kéo dài thân và hình thành lá. Để thu nhận cây con phát triển hoàn chỉnh, những cụm chồi này phải được chuyển sang môi trường không có chất điều hòa sinh trưởng thực vật. Khoảng 26 chồi phát triển bình thường từ mỗi mẫu cấy tTCL sau 2 tuần nuôi cấy. Những chồi khỏe mạnh được chuyển sang môi trường MS có bổ sung 0,4 mg/l NAA kết hợp với 0,2 mg/l Kinetin để kích thích ra rễ. Kết quả nghiên cứu đã chỉ ra rằng việc lựa chọn các chất điều hòa sinh trưởng thực vật là yếu tố chính xác định dạng đáp ứng phát sinh hình thái của mẫu cấy tTCL từ cuống chồi hoa Súp lơ.

Từ khóa: *Brassica oleracea var. botrytis, cuống chồi hoa, TCL, phát sinh hình thái, PGRs*

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