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Curd Formation and Flowering of Plantlets Regenerated from Cauliflower Curd Explants

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In vitro flowering has been reported for several plant species (Scorza, 1982). However, to our knowledge, there have been no reports on in vitro flowering in *Brassica* spp., with the exception of a nonheading Chinese cabbage [*Brassica campestris* ssp. *chinensis* (L.) Makino] commonly known as To-Pe-tsai, from which flowers were obtained from seedling shoot-tip cultures (Shinora et al., 1981). We have observed curd formation and flowering on plantlets regenerated from cauliflower (*B. oleracea* var. *botrytis* L. '60 Day') curd explants. The objectives of this work were to confirm the occurrence of in vitro flowering in cauliflower and to investigate the effect of temperature on flowering.

Curd portions collected from plants grown in the vegetable garden of Zhejiang Agricultural Univ. were cut into small pieces and soaked in tap water plus Tween-20 for 30 min. Curd pieces were soaked in 70% ethanol for 15 to 30 see, rinsed two times in sterile distilled water, soaked in 0.170 mercuric chloride for 3 to 5 rein, and rinsed three more times in sterile distilled water.

Curd tissues were dissected aseptically into 2-to 3-mm-diameter pieces and planted on the surface of agar medium. The medium consisted of MS (Murashige and Skoog, 1962) salts and vitamins, 3% sucrose, and (mg·liter^{-1}) 0.1 N^6-benzyladenine and 1.0 α-naphthaleneacetic acid (Li and Qiu, 1981). The pH was adjusted to 5.8 with 0.1 N HC1 or NaOH before adding agar (0.7%). The medium was dispensed into 50-ml Erlenmeyer flasks (20 ml/flask) and autoclave at 121C for 15 min. Three explants were placed in each flask. Cultures were maintained at 25C and a 12-h photoperiod (46 μmol·m^{-2}·s^{-1}) supplied by cool-white fluorescent tubes.

The explants began to form calli from the cut edges after ≈ 1 week and redifferentiated shoots and roots on the same medium. After 40 days, regenerated shoots could be harvested from the cultures and planted on agar-based MS medium without growth regulators for rooting. The effect of temperature on curd formation was determined by placing the cultures in incubators at 25 or 10C and a 12-h photoperiod (34 μmol·m^{-2}·s^{-1}). This experiment was repeated three times with 20 replicates per treatment.

Plantlets treated at 10 and 25C were transplanted to pots containing 1 perlite : 1 vermiculite (v/v) and kept at 25C to determine their vernalization response. The plantlets grown in vitro at 10C developed complete inflorescences (Table 1), flowered, and formed pods, but most plantlets kept at 25C developed only curds and a few developed rudimentary inflorescences.

Plantlets at 10C had visible curds after 30 days (Fig. 1); at 25C there were no curds, even after 2 months, when the plantlets wilted and died. Plantlets at 10C developed inflorescences in vitro and on transfer to pots.

We have shown that temperature is an important factor in cauliflower curd formation and flowering in vitro and that in vitro-regenerated shoots are in the receptive stage for cold induction. Further studies concerning the mechanism of in vitro curd and inflorescence formation are needed. Since we used a growth-regulator-free medium, our system can be used for studying hormonal regulation of curd formation and flowering in cauliflower.

Fig. 1. Curd formation on in vitro *Brassica oleracea* var. *botrytis* plantlet grown at 10C and then transferred to 25C.

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Table 1. Effect of temperature on growth and development of plantlets from cauliflower curd explants.

	Plantlets[z]			Transplants[y]		
Temp (ºC)	Curd formation (%)	Leaves (no.)	Plantlet ht (cm)	Leaves (no.)	Plant ht (cm)	Inflorescence stalk length (cm)
10+ 25[x]	100	7.0 ± 0.2	4.0 ± 0.4	10.2 ± 1.9	17.2 ± 1.2	11.0± 1.0
25 + 25[w]	0	11.4± 0.6	4.1 ± 0.4	19.3± 1.9	10.8 ± 0.9	4.1 ± 1.0

[z]Data collected after 30 days in vitro.
[y]Data collected 90 days after transplanting to pots.
[x]10C in vitro; 25C after transplanting.
[w]25C in vitro; 25C after transplanting.