Short Communication Plant Tissue Culture Letters, 8(2), 121~123(1991)

Somatic Embryogenesis and Plant Regeneration from *Cyclamen persicum* Mill. Leaf Cultures

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(Received February 21, 1991)
(Accepted March 11, 1991)

Cyclamen (*Cyclamen persicum* Mill.) is an important potted plant of great economic importance in Japan. In horticultural practice, this plant is propagated commercially by seeds, which results in a heterogeneous population of seedlings. Although several attempts have been made to establish clonal propagation using tissue culture, it is still difficult to propagate elite plants in vitro[1].

In this paper, we report successful results in the induction of embryogenic callus and plant regeneration from somatic embryo in a commercial variety of cyclamen.

Embryogenic callus induction

Greenhouse grown *C. persicum* cv. Table Mini Lilac Rose plants of approximately one year of age were used. Three cm long leaves of these plants were rinsed in 70% (v/v) ethanol for 30 seconds, sterilized by immersion for 5 minutes in 5% sodium hypochlorite solution containing 0.01 m*l*/*l* of Tween 80, and rinsed three times in sterilized distilled water. The sterilized leaves were cut into 1 cm diameter pieces with a surgical knife and placed on Linsmaier and Skoog (LS) basal medium[2] supplemented with 3% sucrose, 1 mg/*l* 2, 4-D, and four different concentrations of kinetin (Table 1). The medium was solidified by 0.2% Gelrite. The cultures were incubated in the dark at 26°C.

Callus formation from leaf explants was observed within 20-30 days of culture. Two types of calli were formed: one was transparent and friable and the other was opaque white and compact. Upon transfer to LS growth regulator-free medium, the former calli produced numerous somatic embryos (**Fig. 1a, b**). Table 1 shows the frequency of embryogenic callus formation from leaf explants after 40 days of culture. Embryogenic callus was formed on the media which contained 1 mg/*l* 2, 4-D with

Table 1. Effects of plant growth regulators on embryogenic callus formation from leaf explants.

Growth regulators (mg/*l*)		No. of explants inoculated	Total no. of calli formed	No. of embryo-genic calli
2, 4-D	kinetin			
			%	%
1	0	14	5(35.7)	2(14.3)
1	0.1	15	9(60.0)	7(46.7)
1	0.5	20	20(100)	0
1	1	21	20(95.2)	0

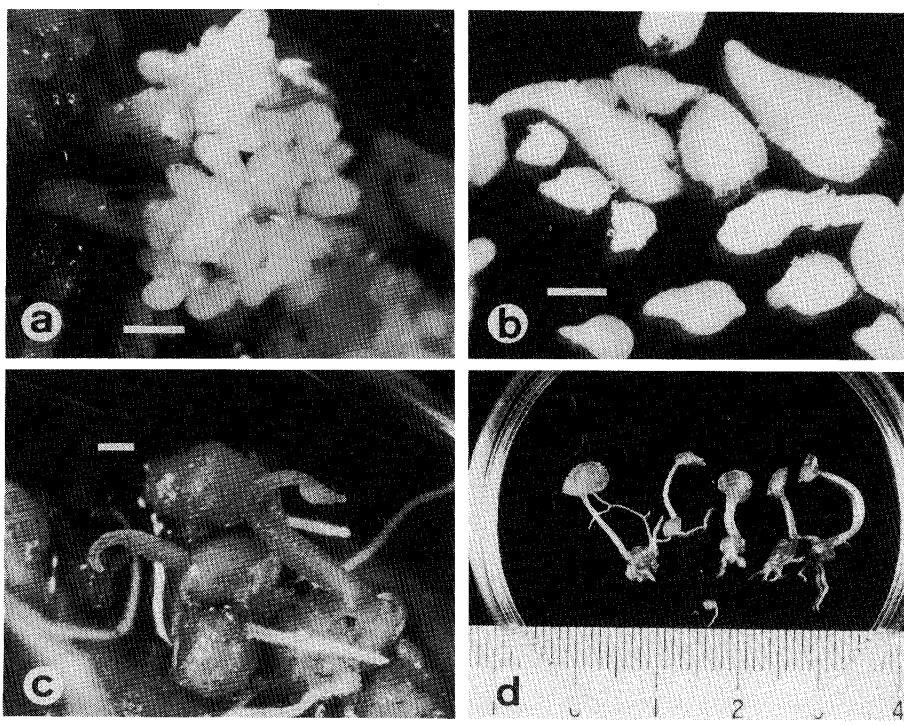

Fig. 1 Samatic embryo formation and plant regeneration from leaf callus cultures of Cyclamen. Bar=1 mm

(a) Somatic embryo formation from leaf callus.
(b) Isolated somatic embryos which varied in the developmental stages.
(c) Tuber formation and shoot and root development on plant regeneration medium.
(d) Shoot and root development from tuber like structures.

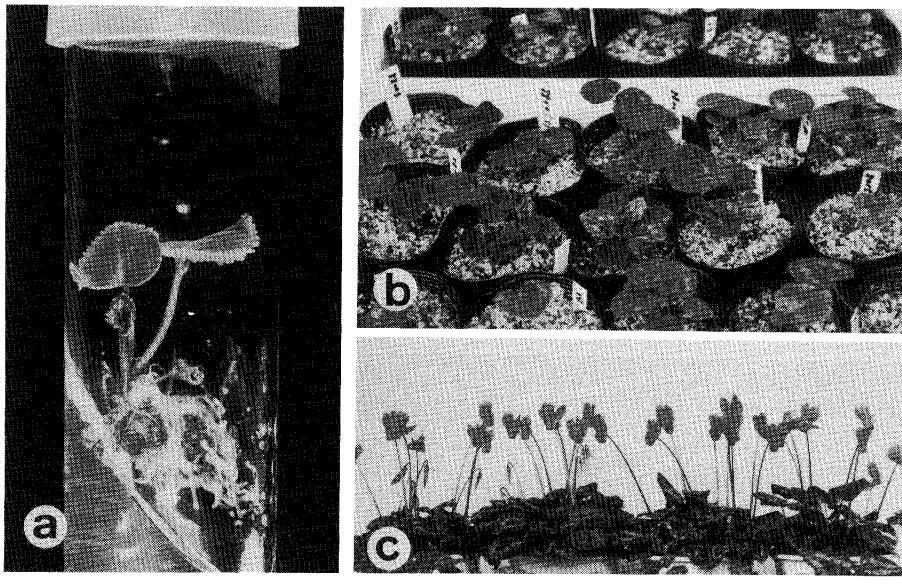

Fig. 2 Cyclamen plants via somatic embryogenesis.

(a) A plantlet growing on plant growth medium.
(b) Plantlets transferred to pots.
(c) Plants that flowered in a greenhouse.

or without 0.1 mg/*l* kinetin. Although kinetin at a low concentration (0.1 mg/*l*) stimulated embryogenic callus formation, at higher concentrations (0.5-1.0 mg/*l*) it suppressed embryogenic callus formation. Embryogenic capacity was retained on LS medium supplemented with 1 mg/*l* NAA, 0.1 mg/*l* kinetin, 5% sucrose and 0.2% Gelrite over succesive subculture.

Plant regeneration from somatic embryo

After 30 days of culturing on LS growth regulator-free medium, somatic embryos formed in leaf calli were transferred to the plant regeneration medium containing half-strength LS salts and vitamins, 1 mg/*l* NAA, 1 mg/*l* kinetin, 5% sucrose and 0.2% Gelrite. Plantlets derived from somatic embryos were transferred to the growth medium containing one-half strength LS medium and 0.2% Gelrite. Somatic embryos and regenerated plantlets were incubated at 26°C and 20°C, respectively, under cool white fluorescent light (6,000 lux) with a 12-hour photoperiod.

Since somatic embryos developed slowly and occasionally ceased to grow on LS growth regulator -free medium, they had to be transferred onto the plant regeneration medium to develop tubers from the embryos. The tubers thus obtained showed close similarity to those derived from seedling tubers (**Fig. 1c, d**). When tubers with buds and roots were transferred to the growth medium, they further developed shoots and roots (**Fig. 2a**). Approximately 90% of them developed into mature plants in a growth chamber, after plantlets of 5 to 10 leaves were transferred to pots (**Fig. 2b**).

Regenerated plants were transferred to pots containing a 2 : 1, vermiculite and perlite mixture. These potted plants were maintained at 20°C under a 12-hour photoperiod (6,000 lux) in a growth chamber. At present, over 200 plants have flowered in a greenhouse (**Fig. 2c**), and have exhibited no morphological variation.

The calli have been embryogenic for four years, and complete plants are still being recovered from them.

References

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《和文要約》

シクラメン葉カルスからの体細胞不定胚形成と植物体再生

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シクラメンの品種 Table Mini Lilac Rose の葉切片からの embryogenic callus を誘導し再分化植物体を得た．embryogenic callus は 1 mg/*l* 2,4-D を単独もしくは 0.1 mg/*l* kinetin を組合わせた LS 培地上で形成された．これらのカルスの不定胚形成能は長期間の継代培養によっても失われず維持された．形成された不定胚を 1 mg/*l* NAA と 1 mg/*l* BA を含む 1/2 LS 培地に移植することによって多数の再分化植物体を得ることができた．