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In vitro Formation of Tuberous Roots from Root Ends in the Rooted Tuberous stem without shoots in *Cyclamen persicum* MILL.

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ABSTRACT

In Japan, propagation of cyclamen is mainly from seedlings. However, seeds are expensive and germination is slow and non-uniform. Therefore, to achieve genetically uniform propagation, multiplication must be vegetative. The rooted tuberous stems without shoots as sources of explants were cultured on the media containing BA and sucrose. After 30 days cultivation, tuberous roots were produced from the root ends attached to a tuberous stem and its capability was dependent on the type of media. The highest percentage of tuberous root formation was observed in culture on the medium of 1/3 MS containing 0.05mgL^{-1}NAA, 0.5 mg L^{-1} BA and 5% sucrose. Growth rates of the tuberous roots were greatly influenced by the cutting positions of a root in explants. The highest growth of was observed if small amount of root end was cut at initiation of tissue culture.

Key words : cyclamen, tuber tissue culture, tuberous root, N-6-Benzyladenine

INTRODUCTION

The genus CYCLAMEN contains 19 species and distributes in the Mediterranean region (Ishizuka and Uematsu, 1992). Plants of this genus have a tuberous stem and elegant nodding flowers. Among them, *Cylamen persicum* MILL. has been cultivated as a commercial plant. Its production in Japan is rapidly increasing and serves as a popular potted plant. Propagation of cyclamen is practically raised from seedlings. However, seeds especially F1 hybrids are very expensive and germination is slow and non-uniform. Therefore, to achieve genetically uniform propagation, multiplication must be vegetative.

Its vegetative propagation is capable not only by the *in vivo* method but also the *in vitro* method. The former methods is achieved by tuber division or propagation from floral trunks (Nakayama, 1980). Reports on the latter methods have presented and proved highly successful for cyclamen multiplication (Geier, 1977, Wainwright and Harwood, 1985). In micro-propagation, it is important to regenerate genetically pure lines, however, somaclonal variations are well known in tissue cultures in cyclamen (Kobayashi, 1992, Karam and Al-Majathoub, 2000). Therefore, propagates by *in vitro* culture must come from direct organogenesis, not though callus generation.

The present study was initiated to assess possibility

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of direct tuberous root regeneration from a root attached to tuberous stem of cyclamen, and the efficiency of different mediums and effect of root cuttings to promote thickening of tuberous roots.

MATERIALS AND METHODS

Plant materials;

The sterilized seeds of cyclamen cv. 'Dressy Scarlet' were germinated on a Murashige and Skoog (MS) medium containing 3% sucrose. The MS medium was adjusted to pH 7.5, and solidified with 6 g/L agar. *In vitro* cultures were maintained for 40 days at 20℃ in the culture room where daylight was maintained for12 hours supplied by white fluorescent lamps at less than 2000 lux.

Preparation of the explants

An aseptic seedling of cyclamen composes of a shoot, a thicken stem that has developed from enlarged hypocotyls tissue and several adventitious roots. After the shoots were cut at the basal portion, seedlings without shoots were used as sources for explants of tissue culture.

***In vitro* culture of the explants**

Such explants of 40 day seedlings were cultured on 1/3 MS medium (concentration of micro-elements of MS as it) and initiated on 20 media containing combinations of N-6-Benzyleadenine (BA) (0, 0.05, 0.1, 0.5 and 1.0 mg/L) and sucrose (3, 5, 7 and 9%). After 30 days cultivation, some explants showed tuberous roots differentiated from the terminal portion of adventitious roots.

In the second experiment, length decreasing treatments on each root at initiation of tissue culture were performed in order to enlarge the newly regenerated tuberous roots from root ends. Cut portions were as follows. Portion 1; without cutting the roots, Portion 2; cutting the place off about 2 to 3 mm from tubers, Portion 3; cutting 1/2 of the full length of the roots, and Portion 4; cutting only about 2 mm of the root ends tip. In this experiment, data of tuberous root formation and their growth rates were collected after 30 days cultivation.

RESULTS AND DISCUSSION

Tuberous root formation

Among herbaceous perennials possessing specialized vegetative structures such as bulb and corm, cyclamen belongs to the tuberous stem group (Hartmann and Kester, 1975). Its plant body is divided into three major portions, distinct from each other in structure and function. These are top shoots, a thickened stem and several adventitious roots. No tuberization occurs on roots in the intact plants.

The 40 day seedlings without shoots employed as sources of explants in the present experiment showed a different organogenesis during 30 days in tissue culture (Table 1). It was influenced with the exogenous growth substances and sucrose present at the time of

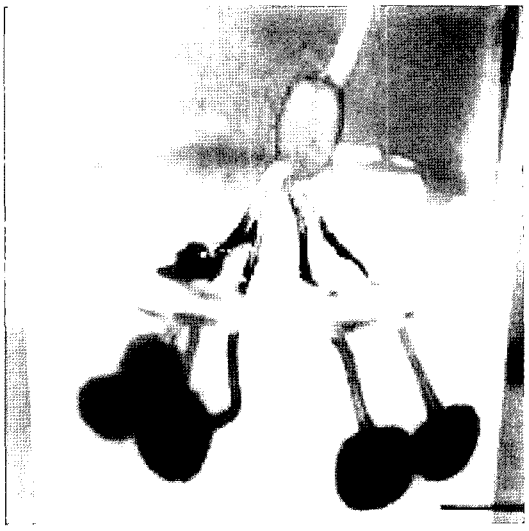

Fig. 1. Induced tuberous roots (root tubers) on root ends of cyclamen seedling after 30 days incubation on the 1/3 MS medium containing 0.05 mg/L NAA, 0.5 mg/L BA and 5% sucrose. The bar represents 5 mm in length.

Table 1. Effect of different concentrations of BA and sucrose on the tuberous root formation of *cyclamen* seedlings .

0.05	3	4.8	0.0	0.00	8.2
0.05	5	5.6	0.0	0.00	21.2
0.05	7	4.0	0.0	0.00	21.4
0.05	9	4.8	0.0	0.00	23.4
0.1	3	4.4	4.2	0.62	0.6
0.1	5	3.4	3.2	0.97	2.0
0.1	7	4.4	4.4	1.12	2.8
0.1	9	3.8	3.8	1.47	3.6
0.5	3	4.2	4.2	1.92	0.0
0.5	5	4.6	4.0	2.60	0.0
0.5	7	5.0	5.0	2.16	0.0
0.5	9	4.0	4.0	1.74	0.0
1.0	3	4.6	4.6	2.52	0.0
1.0	5	5.0	4.8	1.86	0.0
1.0	7	3.4	3.4	1.62	0.0
1.0	9	3.4	3.2	1.34	0.0

*Average of 5 seedlings.

Table 2. Effect of root cutting on enlargement of induced tuberous roots of cyclamen seedlings.

Cut place	No. of seedlings of incubated	Diameter tuberous root (mm)	No. of large one over 3mm in Ø
None	5	1.22	0.0
Cut ca.2-3mm from stem	5	2.51	2.0
Cut 1/2 of full length of root	5	2.62	3.6
Cut ca.2mm from root end	5	3.14	14.2

Result of 30 days incubation

embedding. Microtuberization phenomenon occurred if cultured on media containing combination of 0.1, 0.5 and 1.0 mg/L BA and 3, 5, 7 and 9% sucrose. The largest size of globular tuberous roots (designated as root tubers) was seen on the medium containing 0.05 mg/L NAA, 0.5 mg/L BA and 5% sucrose (Table 1, Fig. 1). Explants kept on BA deficient media did not differentiate tuberous roots, but advantageous to secondary root formation (Table 1). Karam and Al-Majathoub (2000) reported similar results that microtuberization of wild cyclamen occurred if cultivation of root explants on medium of 0.1 /L NAA, 1mg/L BA, and 3% sucrose were performed. The best combination of BA and sucrose concentrations for induction of tuberization would be different among cyclamen cultivars.

Promotion of enlargement of globular tuberous roots by root cuttings

Enlargement of tuberous roots was affected by length decreasing treatments at initiation of tissue culture. All of the tuberous roots differentiated at terminal portion

of roots, and growth rates of them changed according to cut places of roots (Table 2). As compared with the untreated intact roots, the treated roots showed higher growth rates. The greater growth was gained if were cut only about 2 mm of root ends, succeeding the cutting 1/2 of the full length of the roots, and the cutting the portion of about 2 to 3mm from the tuberous stems. As a result, a small amount of cutting of root ends at initiation of tissue culture, stimulated growth of tuberous roots. Diameter of tuberous roots increased three times larger, as compated with intact roots in 30 day culture (Table 2). The larger root tubers were used for succeeding cultures, the easier reproduction of sound nurseries would become in vegetative propagation of cyclamen.

CONCLUSION

The rooted tuberous stems excised shoots of cyclamen cv. 'Dressy Scarlet' started to form tuberous roots on root ends after 30 days culture. This direct tuberous root regeneration was achieved by *in vitro* culture on 1/3 solid MS medium containing 5% sucrose, 0.05 mg/L NAA and 0.5 mg/L BA. To promote satisfactory enlargement of the tuberous roots *in vitro* culture, it would be necessary to excise a small amount of root end tissue at initiation of tissue culture. Further multiplications using directly formed root tubers should be done and cytological research is required to investigate genetic stability of clone lines in cyclamen.

CONSIDERATION

In general, cell division was more powerful as the nearer cutting place effected in a roots.

I think the reason of different promotion of enlargement in the globular tuberous roots by roots cutting was the result of strong enlargement promotion of globular tuberous roots as a nearer roots cutting.

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