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Plantlet Regeneration in Root Segment Culture of Cymbidium Kenny 'Wine Color'

Saburo YASUGI*, Kenji SAKAMOTO*, Kazue ONODERA*
and Meishi TAMASHIRO*

In orchids, shoot tip culture is generally used as a propagation method. However, new shoots must be sacrificed and there is a seasonal limitaion for new shoots. Hence, explants from many other plant parts, such as flower stalk[1], pseudobulb[9] and leaf[11], have been successfully utilized. Root explant was also used as an useful material to get new plants without sacrificing new shoots. Root or rhizome explants have successfully been used for the propagation of some orchids such as *Catasetum*[6], *Eulophia*[10], *Cyptopodium*[7], *Mormodes*[2-5], *Rhynchostylis*[8], *Bletilla, Cleisostoma* and *Pholidota*[12].

The purpose of this study is to show the possible use of root culture as one of the propagation methods in *Cymbidium*, which is a very popular orchid in Japan.

Roots (about ϕ 2×30 mm) of *Cymbidium* Kenny 'Wine Color' were excised from the mericlone plantlets grown *in vitro* for one year, and cut into three parts (tip, middle and basal). Roots of each part were cut into 5 mm-long segments. To examine the effects of plant growth regulators on the PLB formation a total of 48 root segments were cultured on the basal MS medium (pH 5.8) supplemented with 1 m*l*/*l* Hyponex (5-10-5) solution, 2% sucrose, 8 g/*l* agar and a combination of NAA and BA at 0 or 1 mg/*l* under continuous light (220 lux) or dark condition at 22°C.

After 8 weeks of culture, many root segments survived and the highest survival rate was observed in the basal segment of root under light condition (**Table 1**). On the contrary, the tip part of root segments elongated vigorously as root (**Fig. 1-a**).

PLBs were induced only when the basal segments of root were cultured on a medium containing 1 mg/*l* NAA and 1 mg/*l* BA under light condition (**Table 1, Fig. 1-c**). Under this culture condition, PLB began to be formed at 2 weeks of culture and the PLB formation rate was 29.2% at 15 weeks. Plantlets were produced from all 14 PLBs within 6 weeks after transfer onto the medium without plant growth regulators (**Fig. 1-d**).

In the previous report on the root culture, light promoted root tip growth in *Pholidota*, whereas

Table 1. The rates of survival, root elongation and PLB formation of segments in three parts (tip, middle and basal) of root cultured for 8 weeks under light or dark condition.

Hormones	Rates of [survival (%); elongation (mm); PLB formation (%)]*1					
NAA, BA	Light condition			Dark condition		
(ppm)	Tip	Middle	Base	Tip	Middle	Base
0, 0	[100; 5.4; 0]	[80; 0.1; 0]	[100; 0.1; 0]	[80; 8.7; 0]	[100; 0.2; 0]	[60; 0.2; 0]
1, 0	[100; 2.7; 0]	[60; 0.2; 0]	[100; 0.2; 0]	[100; 10.0; 0]	[80; 0.2; 0]	[100; 3.7; 0]
0, 1	[80; 0.8; 0]	[100; 0.0; 0]	[100; 0.1; 0]	[100; 6.7; 0]	[60; 0.0; 0]	[80; 0.0; 0]
1, 1	[100; 2.1; 0]	[100; 0.3; 0]	[100; 0.0; 16.7]	[100; 0.0; 0]	[100; 0.0; 0]	[100; 0.0; 0]

*Department of Horticulture, University of Minamikyushu, Hibarigaoka, Takanabe-cho, Miyazaki 884 Japan

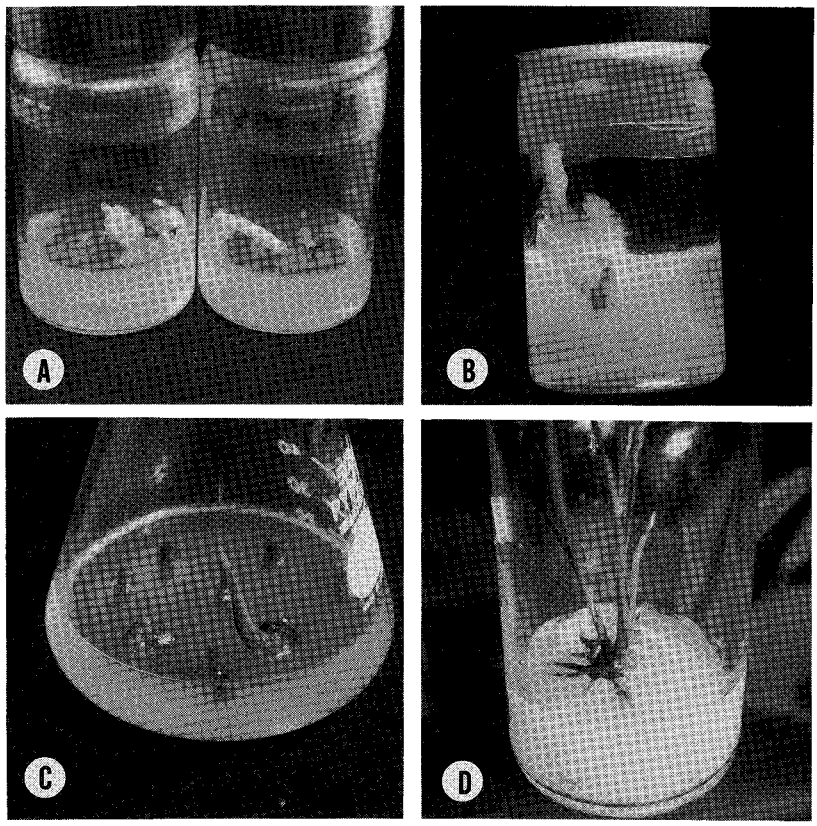

Fig. 1 Plantlet regeneration from root segments of *Cymbidium* Kenny 'Wine Color'.
(A and B) Elongation of root tip segments under light (A) and dark (B) conditions after 8 weeks of culture. (C) PLB and shoot formed from basal segments under light condition after 12 weeks of culture. (D) A plantlet regenerated from root segment after 25 weeks of culture.

Bletilla preferred darkness[12]. In this study, darkness promoted only elongation of root segment (**Fig. 1-b**), whereas light was essential for inducing PLB.

In *in vitro* culture of root or rhizome segments culture of orchids, shoot or PLB formation was promoted by adding NAA and BA in *Bletilla, Cleisostoma*[12] and *Mormodes*[4]. Depending on the concentrations of NAA and BA, different morphogenetic responses such as root growth or differentiation of PLB were observed in *Cyrtopodium*[7]. In this study, only the treatment with 1 mg/*l* NAA and 1 mg/*l* BA induced PLB formation in *Cymbidium*.

In this experiment, only basal segments of root produced PLBs. Hölters and Zimmer[3] suggested that the sites of PLB formation on the axis of the root corresponded to those of natural lateral root formation. Therefore, it seems that PLB was also formed from the primordium of lateral root in the basal part of root in *Cymbidium*.

In conclusion, the root segment culture in *Cymbidium* Kenny 'Wine color' is proved as an useful method to induce PLB and plantlets, and it is essential to culture the basal segments of root on a medium contanining NAA and BA under light condition. (Accepted February 12, 1994)

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