

# Effects of Basal Medium and Concentration of Sugar and Banana Flesh on the Growth of *Oncidium* Protocorm-like Bodies Cultured in Vitro

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## INTRODUCTION

We have reported previously that the growth of in vitro plantlets of *Cymbidium* and *Cattleya* was promoted remarkably when organic supplements were added to the basal medium (Kusumoto, 1969; Kusumoto and Furukawa, 1977; Kusumoto, 1979a; Kusumoto, 1979b; Kusumoto, 1980). In our last report we described the effects of basal medium and the concentration of added sugar or banana flesh on the growth of *Oncidium* plantlets cultured in vitro (Kusumoto and Takeda 1997). In the present experiment, the effects of the same factors as the last experiment were investigated on the growth of separated *Oncidium* protocorm-like bodies (PLB) which were not cut up.

## MATERIALS AND METHODS

The PLB were used as the experimental material and were obtained by in vitro culture of peduncles of axillary buds of *Oncidium* 'Aloha Iwanaga'. Each small PLB, 2.5 mm in diameter, without differentiated buds and roots was selected by separation, and 40 pieces (1 g in total weight) of selected PLB were planted on each 50 ml of medium which was contained in a 200-ml flask. Murashige and Skoog's (MS) nutrient medium was used as the standard basal medium, with 0.1 mg liter<sup>-1</sup> of NAA and BA, 3 g liter<sup>-1</sup> of Gellan gum, and 20 g liter<sup>-1</sup> of sucrose added to the basal medium. The PLBs were cultured under 16-h day length at 2500 lx and 24±2°C. PLB growth was checked at 60 days after planting.

**Experiment 1.** Four different media, MS, White(W), Knudson's C (KC), and Hyponex (H), were used as basal media in this experiment. In addition to these basal media, MS medium at ¾ and ½ normal concentration was investigated.

**Experiment 2.** Banana flesh (100 g liter<sup>-1</sup>) was added in each basal medium to check its effects on the growth of the PLB.

**Experiment 3.** Sugar and banana flesh concentrations were varied to check effects on growth and differentiation. In this experiment the PLB, with buds over 1 cm in height and roots were defined as differentiated PLB, smaller were classified as undifferentiated.

## RESULTS AND DISCUSSION

The results are shown in Tables 1, 2 and 3. The results from Experiment 1 showed that the most suitable basal medium for the growth of the PLB was MS as in the earlier experiment. At the lower MS medium concentrations (¾ MS and ½ MS)

**Table 1.** Effects of basal medium and concentration of Murashige and Skoog medium.

Medium	Growth index	Fresh weight of dif. (g)	Fresh weight of undif. (g)**	No. of dif.**	Aver. height of shoot (cm)	Aver. no. of roots	Aver. length of roots (cm)
MS	33.7	5.3	28.4	22	2.4	2.1	1.4
¾MS	30.6	3.2	27.4	16	2.4	2.1	1.9
½MS	28.9	3.0	25.9	12	2.2	2.3	2.3
W	10.5	0.0	10.5	0	0	0	0
KC	15.0	0.0	15.0	0	0	0	0
H	24.1	0.6	23.5	3	2.0	2.7	2.3

\* Growth index = growing total fresh weight/planting total fresh weight.

\*\* Fresh weight of dif. = total fresh weight of differentiated PLB.

Fresh weight of undif. = total fresh weight of undifferentiated PLB.

No. dif. = number of differentiated PLB.

\*\*\*PLB = protocorm-like bodies, MS = Murashige and Skoog, W = Whites

**Table 2.** Effects of basal medium, Murashige and Skoog medium concentration and addition of banana flesh on the growth of *Oncidium* PLB cultured in vitro.

Medium***	Growth index	Fresh weight of dif. (g)	Fresh weight of undif. (g)**	No. of dif.**	Aver. height of shoot (cm)	Aver. no. of roots	Aver. length of roots (cm)
MS	33.7	5.3	28.4	22	2.4	2.1	1.4
MSB-100	43.5	15.0	28.5	40	3.5	2.9	2.2
¾MSB-100	34.7	11.4	23.3	34	3.6	2.7	3.1
½MSB-100	29.1	9.5	19.6	28	2.6	3.2	3.1
WB-100	13.9	1.9	12.0	5	1.7	2.1	3.4
KCB-100	26.9	9.6	17.3	29	2.2	2.8	2.6
HB-100	25.9	11.3	14.6	32	2.1	2.3	3.3

\* Growth index = growing total fresh weight/planting total fresh weight.

\*\* Fresh weight of dif. = total fresh weight of differentiated PLB.

Fresh weight of undif. = total fresh weight of undifferentiated PLB.

No. dif. = number of differentiated PLB.

\*\*\*PLB = protocorm-like bodies, MS = Murashige and Skoog, B = banana, W = Whites medium, K = Knudson's, and H = Hyponex.

**Table 3.** Effects of Murashige and Skoog medium containing various concentrations of sugar or banana flesh on the growth of *Oncidium* PLB cultured in vitro.

Medium***	Growth index	Fresh weight of dif. (g)	Fresh weight of undif. (g)**	No. of dif.**	Aver. height of shoot (cm)	Aver. no. of roots	Aver. length of roots (cm)
MS(S-20)	33.7	5.3	28.4	22	2.4	2.1	1.4
MSS-30	37.1	7.1	30.0	40	2.5	2.5	1.3
MSS-40	40.8	12.9	27.9	40	3.2	4.0	2.0
MSB-100	43.5	15.0	28.5	40	3.5	2.9	2.2
MSB-150	36.4	11.0	25.4	32	2.9	2.3	3.1
MSB-200	29.1	9.5	19.6	14	2.2	2.9	3.3

\* Growth index = growing total fresh weight/planting total fresh weight.

\*\* Fresh weight of dif. = total fresh weight of differentiated PLB.

Fresh weight of undif. = total fresh weight of undifferentiated PLB.

No. dif. = number of differentiated PLB.

\*\*\*PLB = protocorm-like bodies, MS = Murashige and Skoog, S = sucrose, and B = banana.

growth index, weight, and number of differentiated PLB decreased, but differentiation and elongation of roots was promoted. Medium H was slightly inferior in growth index, markedly inferior in the number of differentiated PLB to those on  $\frac{1}{2}$  MS, but the same as  $\frac{1}{2}$  MS in root development of differentiated PLB. When medium W was used, the green color of the PLBs changed to yellow and growth and height decreased. No differentiated PLBs occurred in media W and KC. We believe that the salt concentrations of the W and KC media were too low and insufficient when compared with the MS medium, i.e., the high salt level in the MS medium was better suited to the growth of *Oncidium* PLB. These results were the same as with the growth of *Oncidium* plantlets in the previous experiment.

The results from Experiments 1 and 2 showed that the addition of 100 g liter<sup>-1</sup> of banana flesh to each medium considerably promoted the growth of PLB. In Experiment 3, the addition of sucrose at 1.5 times (30 g liter<sup>-1</sup>) and 2 times (40 g liter<sup>-1</sup>) that of the standard MS medium promoted the growth of PLBs as did the addition of 100 g liter<sup>-1</sup> of banana flesh. Increasing the quantity of banana flesh above 100 g liter<sup>-1</sup> in the medium retarded the growth of PLB after planting, and decreased the growth index, number and weight of differentiated PLB, but promoted root elongation. Our conclusion is that *Oncidium* PLB in vitro exhibited the best growth with the addition of 40 g liter<sup>-1</sup> of sucrose and 100 g liter<sup>-1</sup> of banana flesh to the MS medium. These results are similar to those noted with *Oncidium* plantlets (previous report), but it differs in the amounts of sugar or banana flesh required. With PLB the range of sugar content is wider and that of banana flesh is narrower in *Oncidium* in comparison to plantlets cultured in vitro. The optimum content of sugar or banana flesh differs between PLB and plantlets as was found previously with *Cattleya* and *Cymbidium*.

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