

Effect of Hyaluronic Acid on Organogenesis in Rhizome Cultures of *Cymbidium kanran* Makino

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The effects of adding two types of hyaluronic acid with different molecular weights (HA9, M.W. 8 - 11.7 KDa; HA12, M.W. 11 - 16 KDa; Shiseido Co. Ltd., Japan), to modified MS on organogenesis in rhizome-tip cultures of *Cymbidium kanran* was investigated and compared with adding BA and NAA. HA9 was most effective in inducing shoot formation. The results suggested that HA9 is effective for rhizome and shoot formation. HA12 is effective for rhizome-like shoot formation. BA and NAA did not have a significant effect on regulating organogenesis in this species. It was demonstrated that HA9 and HA12 could be used as plant growth regulators for rhizome-tip cultures of a terrestrial *Cymbidium* species.

Cymbidium kanran is a terrestrial species and known as a temperate species which is difficult to propagate (Shimasaki and Uemoto 1990). Several studies have reported cytokinin-induced shoot formation in rhizome cultures of *C. kanran* and closely related species (Hasegawa et al. 1985, Hasegawa and Goi 1987, Kokubu et al. 1980, Lee and So 1985, 1988, Lee 1988.).

We investigated the effect of hyaluronic acid with different molecular weights (HA9 and HA12, Shiseido, Japan) on organogenesis in rhizomes of *Cymbidium kanran* cultures *in vitro* and compared it with BA and NAA supplemented with modified MS.

C. kanran rhizome proliferated in modified MS excised rhizome apical segments (ca. 5 mm in length), served as explants. UM culture bottles (250 ml) containing 30 ml of medium were used in the experiments. Fifteen rhizome segments were cultured in each treatment. Cultures were maintained at $25 \pm 1^\circ\text{C}$

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under white fluorescent light at 25 $\mu\text{E}/\text{m}^2/\text{s}$ for a 16 hrs photoperiod for 8 weeks. Experimental data were collected after 8 weeks.

The effect of HA9 on rhizome growth and shoot formation is shown in Table 1 and Fig. 1A. The highest rate of rhizome branch formation was 93.3% and the maximum rhizome number (3.4) was recorded in the medium containing 0.01 mg/l HA9. Adding HA9 0.1 and 1 mg/l resulted in the highest number of shoots (53.3%). The effect of HA12 on organogenesis in rhizomes is shown in Table 2 and Fig. 1B. The highest rate of rhizome branch formation was 73.3% and the maximum rhizome number (3.4) was recorded in the medium containing 0.01 mg/l, 0.1 mg/l, and 1 mg/l HA12. The shoot formation was (30%) and shoot number (0.4) for the control treatment. The effect of BA and NAA on rhizome and shoot branch formation is shown in Table 3 and Fig. 1C. The media supplemented with BA and NAA showed the maximum number of rhizomes

Table 1. Effect of HA9 on organogenesis in rhizome culture of *C. kanran* after 8 weeks.

HA9 (mg/l)	Rhizome		Shoot-like rhizome		Shoot		Rhizome-like shoot	
	Average number	Form. rate (%)	Average number	Form. rate (%)	Average number	Form. rate (%)	Average number	Form. rate (%)
0	1.7 \pm 0.5	50.0	0.2 \pm 0.1	10.0	0.4 \pm 0.1	30.0	1.2 \pm 0.5	50.0
0.01	3.4 \pm 0.3	93.3	1.6 \pm 0.2	86.6	0.6 \pm 0.2	40.0	3.4 \pm 0.4	93.3
0.1	2.9 \pm 0.6	80.0	1.7 \pm 0.3	80.0	0.5 \pm 0.1	53.3	2.6 \pm 0.4	86.6
1	3.2 \pm 0.6	80.0	1.7 \pm 0.4	80.0	0.5 \pm 0.1	53.3	3.0 \pm 0.8	80.0
10	1.6 \pm 0.4	73.3	1.1 \pm 0.2	80.0	0.6 \pm 0.4	33.3	1.5 \pm 0.3	80.0

Values represent means \pm S.E. The cultures were examined after 8 weeks. Each treatment consisted of 3 replicates, and each replicate consisted of 5 rhizomes.

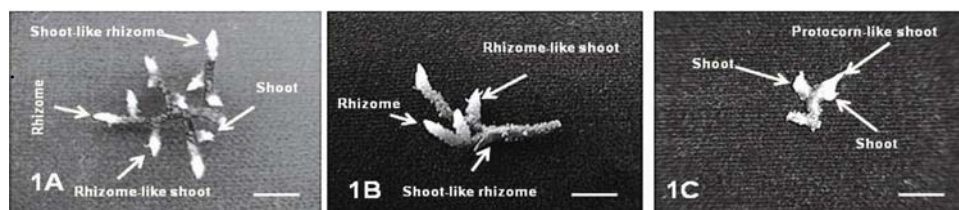


Fig. 1. The effect of HA9, HA12 and combination of BA and NAA on organogenesis in rhizome cultures 1A : HA9 0.01 mg/l, 1B : HA12 0.01 mg/l and 1C: BA 0.1mg/l + NAA 0.1 mg/l. (Bars =10 mm).

(60%) and the maximum rhizome number (1.7) was obtained at 1 mg/l BA, 0.1 mg/l NAA, 0.1 mg/l BA + 0.1 mg/l NAA, and 1 mg/l BA + 0.1 mg/l NAA. The maximum shoot formation (46.6%) and the maximum number of shoots (0.9) was recorded in the combined treatment of 0.1 mg/l BA and 0.1 mg/l NAA.

The rate of formation of rhizome branches, shoots, shoot-like rhizomes and rhizome-like shoots was higher in HA9 than HA12 or the combined application of BA and NAA. These results indicate that HA9 can stimulate rhizome induction. Low molecular weight HA9 was more effective for growth promotion than that of higher one.

Table 2. Effect of HA 12 on organogenesis in rhizome culture of *C. kanran* after 8 weeks.

HA 12 (mg/l)	Rhizome		Shoot-like rhizome		Shoot		Rhizome-like shoot	
	Average number	Form. rate (%)	Average number	Form. rate (%)	Average number	Form. rate (%)	Average number	Form. rate (%)
0	1.7 ± 0.5	50.0	0.2 ± 0.1	10.0	0.4 ± 0.1	30.0	1.2 ± 0.5	50.0
0.01	3.4 ± 0.9	73.3	0.7 ± 0.1	60.0	0.2 ± 0.1	26.6	2.5 ± 0.6	60.0
0.1	1.5 ± 0.3	73.3	0.6 ± 0.2	46.6	0.1 ± 0.1	13.3	1 ± 0.2	60.0
1	1.8 ± 0.3	73.3	0.7 ± 0.1	60.0	0.2 ± 0.1	13.3	1.1 ± 0.2	66.6
10	1.4 ± 0.6	50.0	0.4 ± 0.2	20.0	0.1 ± 0.1	10.0	1.2 ± 0.5	40.0

Values represent means ± S.E. The cultures were examined after 8 weeks. Each treatment consisted of 3 replicates, and each replicate consisted of 5 rhizomes.

Table 3. Effect of BA and NAA added to MS on rhizome and shoot formation rhizome culture of *C. kanran* after 8 weeks.

Treatments (mg/l)		Rhizome		Shoot-like rhizome		Shoot		Rhizome-like shoot	
BA	NAA	Average number	Rate (%)	Average number	rate (%)	Average number	rate (%)	Average number	rate (%)
0	0	1.7 ± 0.5	50.0	0.2 ± 0.1	10.0	0.4 ± 0.1	30.0	1.2 ± 0.5	50.0
0.1	0	0.7 ± 0.1	53.3	0.4 ± 0.1	40.0	0	0.0	0.2 ± 0.1	20.0
1	0	1.0 ± 0.3	60.0	0.4 ± 0.1	40.0	0.9 ± 0.4	33.3	0.2 ± 0.1	26.6
0	0.1	0.8 ± 0.2	60.0	0.4 ± 0.2	33.3	0	0.0	0.4 ± 0.1	33.3
0.1	0.1	1.6 ± 0.5	60.0	0.6 ± 0.2	40.0	0.8 ± 0.2	46.6	0.6 ± 0.2	26.6
1	0.1	1.0 ± 0.2	60.0	0.7 ± 0.1	60.0	0.8 ± 0.3	40.0	0.4 ± 0.1	40.0
0	1	0.8 ± 0.3	46.6	0.6 ± 0.2	40.0	0	0.0	0.2 ± 0.1	13.3
0.1	1	0.7 ± 0.2	46.6	0.3 ± 0.1	26.6	0.6 ± 0.3	20.0	0.4 ± 0.1	33.3
1	1	1.0 ± 0.3	46.6	0.4 ± 0.1	40.0	0.4 ± 0.1	26.6	0.3 ± 0.1	33.3

Values represent means ± S.E. The cultures were examined after 8 weeks. Each treatment consisted of 3 replicates, and each replicate consisted of 5 rhizomes.

In terrestrial *Cymbidium* cultures, higher concentrations of BA added to culture medium promote shoot formation (Shimasaki and Uemoto 1990). Nahar et al. (2011) showed hyaluronic acid can be applied to epiphytic *Cymbidium* species. Present results suggest that HA9 and HA12 can be used as a growth

regulator for terrestrial *Cymbidium* cultures. Chitosan added to culture media promotes organogenesis in *Cymbidium* species (Shimasaki et al. 2003). Our results show that hyaluronic acid can be used as a new plant growth regulator for terrestrial *in vitro* culture of *Cymbidium*.

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