Journal of Life Science 2007 Vol. 17. No. 10. 1336~1340 ©JLS / ISSN 1225-9918

Efficient Bulblet Regeneration and Growth from Bulb Scale of *Hyacinthus orientalis* L. cv. Pink Pearl Cultured *in vitro*

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Received September 14, 2007 / Accepted October 8, 2007

The regeneration and growth of bulblets from the bulb scale segments of *Hyacinthus orientalis* L. cv. Pink Pearl were more efficient in IBA than IAA at the same concentrations (1.0 and 3.0 mg/l). The normal (base-down) orientation of explants was more effective for bulblet regeneration and root growth than the inverted (base-up) orientation. The growth of bulblets and roots was increased higher in the perlite than the agar medium. These results suggested that the alternate culture system, first cultured in the agar medium for bulblet regeneration, and then in the perlite medium for bulblet growth, may be more useful for efficient *in vitro* culture of hyacinth (*H. orientalils*) cv. Pink Pearl.

Key words – Bulblet regeneration and growth, explant orientation, *Hyacinthus orientalis*, IBA, perlite

Introduction

Hyacinth (*Hyacinthus orientalis* L.) is one of the most important ornamental bulb plants. It is so popular in house and garden culture that over 2,000 cultivars have been cultivated and named, according to various flower colours such as white, pale yellow, pink, red or purple. Most hyacinth cultivars can grow in a wide range of environmental conditions. Although hyacinth species have the cultural advantages of tolerating various environmental conditions, the natural production rates of their bulblets for multiplication are very low and the number of the bulblets developed in the scale segments is also very small [1,9].

To improve their propagation ability, in vitro culture techniques were introduced and, as a result, various *in vitro* culture systems were established. It has been known that the *in vitro* multiplication of hyacinth bulblets has been depended on some factors such as plant growth regulators, explant sources, genotypes, temperatures, sucrose concentrations, etc [1,9,12,19,20].

Bulb scales are most commonly used as explants for *in vitro* micropropagation of geophytes [11]. In this study, we have investigated the effects of three factors on bulblet regeneration and growth from bulb scale segments of the cultivar Pink Pearl. These factors are treatments of IAA and IBA generally used for bulblet formation [3,6,13,15], the orientation of explants [12,14,19,20], and the use of perlite as an alternative medium to agar. Although agar is most effective for the support of hyacinth explants, when their regenerated bulblets or plantlets are transferred to other culturing materials such as potting or soils, some difficulties with handling are frequently encountered [8, 16-18].

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Materials and Methods

Plant materials

Bulbs of *Hyacinthus orientalis* L. cv. Pink Pearl, whose diameters were 5.5~6.0 cm, were obtained from a commercial seed company (*ZaboPlant*, Korte Belkmerweg, The Netherlands) and maintained at 20°C after their purchase, and used when shoots developed to approximately 1.0 cm. After the bulbs were sterilized as previously described [19], they were longitudinally cut into 8 pieces and then their outermost scales removed. The bulb scale segments with approximately equal sizes were prepared by removing the outermost scale layers from the bulb scale segments and the detailed procedures were carried out according to the method of Yi et al. [19].

Bulblet regeneration and growth

The bulb scale segments were established in vitro in a modified Heller's medium [5] containing (per liter); 12.5 mg NaFe-EDTA, 1.0 mg glycine, 0.25 mg nicotinic acid, 0.25 mg pyridoxine, 0.05 mg thiamine-HCl, 50 mg myo-inositol and 2% sucrose. Four auxin treatments IAA 1.0 mg/l, IAA 3.0 mg/l, IBA 1.0 mg/l or IBA 3.0 mg/l were individually supplemented to the above medium. Before 0.6% (w/v) agar were added to all the media, the pH was adjusted to 5.4 with 1 N NaOH and then they were melted. Approximately 13 ml of the melted agar medium was poured into a culture tube with the size of 22 x 160 mm and autoclaved [19].

The sterile scale segments were prepared as mentioned above and placed into the above media to a depth of 3 mm and oriented base-down (normal) or base-up (inverted). To analyze the practical use of perlite as an alternative to agar, about 13 g of the aseptic perlite saturated with the sterile media containing the auxins mentioned above were packed into the culture tubes. Then the vigorous bulblets whose diameter reached about 5.0 mm were selected after 16 weeks of culture and transferred to the culture tubes packed with the saturated perlite or solidified with agar.

The cultures for bulblet regeneration were incubated in the dark condition and the regenerated bulblets were subcultured under cool-white fluorescent lamps (Toshiba Fl40 SW) of 30~50 $\mu molm^{-2}s^{-1}$ for 16/8 hr photoperiods of light/dark, at 23±1°C for 16 weeks.

For the quantitative measurements of the percent of bulblet regeneration and root formation, the mean values were calculated from 5 replications, each replication had 20 explants.

Results and discussion

Effect of auxin and explant orientation on bulblet regeneration and root formation

The bulblet regeneration frequency of *H. orientalis* L. cv. Pink Pearl was highest on a concentration of 1.0 mg/l IBA with the normal orientation of the explants. In contrast, the lowest was observed in 3.0 mg/l IAA in the inverted orientation of the explants. However, with the exception of 1.0 mg/l IAA, the bulblet regeneration was relatively high in explants placed in the normal orientation (Fig. 1A). These results indicate that the explant orientation might influence

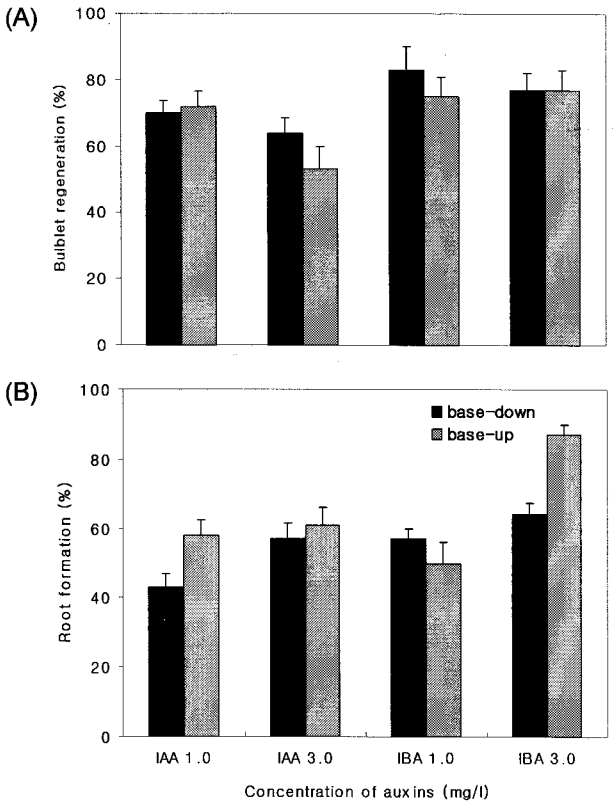

Fig. 1. Effect of auxin treatment and explant orientation on bulblet regeneration and root formation from bulb scale segments of *H. orientalis* L. cv. Pink Pearl after a 16-week culture. Each value represents the mean from 20 bulb scale explants. Experiments were performed five times, and the error bars indicate standard errors for five sets of the results.

on the bulblet regeneration of cv. Pink Pearl.

The root formation frequency of cv. Pink Pearl was the highest in 3.0 mg/l IBA in the inverted orientation of the explants, while the lowest was with 1.0 mg/l IAA in the normal orientation of the explants. The rates of the root formation were relatively high at a high level of IAA or IBA (3.0 mg/l) and increased in IBA more than IAA. However, with the exception of 1.0 mg/l IAA, relatively higher root formation was observed in explants placed in the inverted orientation (Fig. 1B). These results also showed that the explant orientation might have an effect on the root formation of cv. Pink Pearl. Some reviews have reported that most hyacinths do not require growth regulators for bulblet regeneration [6,7,20], others reported that addition of auxin [3,21] promotes their regeneration. Accordingly, their reports indirectly support our results although plant materials and culture conditions used were different.

Effect of auxin and explant orientation on bulblet and root growth

To investigate the effects of the auxins and explant orientation on their growth, the growth of bulblets and roots formed from the bulb scale segments of cv. Pink Pearl were analyzed by measuring the height and diameter of the bulblets, and the length and number of the roots per explant, respectively (Fig. 2A,B).

The blublet growth were slightly increased in 1.0 mg/l IAA or 3.0 mg/l IBA with the normal orientations of the explants. However, there was no significant difference in effect of gowth regulator and explant orientation between them. On other hand, the greatest root growth was observed in 3.0 mg/l IBA with the normal orientation of the explants. The growth of roots length was increased efficiently by IBA and the normal orientation of the explants (Table 1). These results were similar with those of cv. Carnegie [20]. However, these results make no great difference between gowth regulator and explant orientation.

The bulblet formation from the bulb scale segments was detected on dorsal side on any explant orientation and most bulb scale explants were with one bulblet (Fig. 2A,B). However, there was no significant difference in effects of gowth regulator, explant orientation and the origin of bulblet regeneration among them (data not shown). It has generally been known that the bulblet formation from hyacinth scales is initiated at their base due to their basipetal polarity [15], the normal orientation may be more effective for bulblet formation and root growth than the inverted orientation. Nonetheless, several workers reported that when the explants were placed in the inverted orientation, their bulblet regeneration and growth were considerably enhanced [4,13,14]. However, our results are inconsistent with these reports.

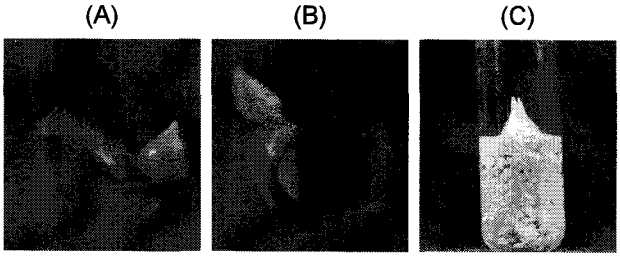

Fig. 2. Bulblet regeneration in bulb scale segments of *H. orientalis* L. cv. Pink Pearl. (A) Bulblet regenerated from a explant of the normal (base-down) orientation; (B) Bulblet regenerated from a explant of the inverted (base-up) orientation; (C) Bulblet transferred in the perlite medium as an alternative to agar. The arrow shows a dorsal side of a bulb scale segment.

Effects of perlite and agar on the growth of bulblets and roots

The well-grown bulblets (about 0.6~0.8 cm high and 0.3~0.5 cm wide) with roots (about 0.2~0.4 cm long) cultured for 16 weeks were selected and transferred to either the agar-solidified medium (agar medium) or the medium containing perlite saturated with the liquid medium (Fig. 2C). The media were supplemented only with 1.0 and 3.0 mg/l IBA because IBA was more effective for the bulblet regeneration and growth than IAA (Table 2).

After transfer, the overall bulblet growth in the perlite medium were enlarger than in the agar medium. In root growth, the perlite medium was also more efficient. Particularly, the root number per explant was great increase in the media adding 1.0 mg/l IBA (Table 2). These results suggest that the use of perlite could be recommended for support of hyacinth explants as an alternative to agar in their in vitro culture.

Agar has been generally used as a material for supporting

Table 1. Effect of auxin and explant orientation on bulblet regeneration from bulb scale segments of *H. orientalis* L. cv. Pink Pearl after a 16-week culture[z]

Growth regulators (mg L^{-1})	Bulblet				Root			
	Height (cm)		Diameter (cm)		Length (cm)		Number	
	Down[y]	Up[x]	Down	Up	Down	Up	Down	Up
IAA 1.0	1.0±0.1	0.9±0.1	0.6±0.1	0.5±0.1	0.9±0.1	0.5±0.1	2.0±0.1	1.5±0.1
IAA 3.0	1.0±0.1	1.0±0.1	0.5±0.0	0.5±0.1	0.7±0.1	0.5±0.1	2.0±0.2	2.0±0.1
IBA 1.0	1.0±0.1	0.8±0.1	0.5±0.1	0.5±0.1	1.2±0.1	0.6±0.1	1.4±0.1	1.9±0.2
IBA 3.0	1.0±0.1	0.8±0.1	0.6±0.1	0.5±0.1	1.7±0.3	0.7±0.1	2.5±0.2	1.7±0.1

[z]Each value represents the mean ± SE of 8 bulb scale explants. The experiment was performed five times with similar results.
[y]The bases of explants were placed in the medium in downward orientation.
[x]The bases of explants were placed in the medium in upward orientation.

Table 2. Comparision between the effects of agar and perlite media on the growth of bulblets and roots subcultured for 16 weeks[z]

Support	Growth regulator (mg L^{-1})	Bulblet		Root	
		Height (cm)	Diameter (cm)	Length (cm)	Number
Agar	IBA 1.0	1.1±0.2	0.4±0.1	0.4±0.1	3.3±0.2
	IBA 3.0	1.0±0.1	0.4±0.1	0.4±0.1	2.2±0.3
Perlite	IBA 1.0	1.3±0.2	0.6±0.1	0.6±0.1	4.8±0.4
	IBA 3.0	1.2±0.2	0.7±0.1	0.6±0.1	3.0±0.3

[z]Each value represents the mean ± SE of 20 bulblets.

explants in vitro culture of geophytes [11]. However, when the explants are transferred for their subculture or plantlets are transplanted to soils, their roots are frequently damaged as the agar attached to their roots are removed, sometimes resulting in the complete failure of growth. Vishnevetsky et al. [17] reported that small bulblets of nerine cultured in liquid medium grew into larger bulbs faster than those cultured on semi-solid medium. Accordingly, perlite may be more efficient than agar because perlite is convenient for handling the explants or the plantlets formed in vitro culture, although our results showed that there was no very great difference between the effects of perlite and agar medium on the bulblet and root growth.

As a result, it could be cautiously concluded that the culture system developed in this study may be more useful for efficient in vitro culture of hyacinth bulb scale. In the first step for bulblet regeneration, the explants are cultured in the agar medium supplemented with 1.0~3.0 mg/l IBA in base-down(normal) orientation. Then in the second steps for bulblet growth, the bulblets with roots are cultured in the perlite medium with the same concentrations of IBA.

Acknowledgement

This work was supported by the Dong-A University Research Fund in 2005.

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초록 : 히아신스(cv. Pink Pearl)의 인편 기내 배양시 효과적인 자구의 재생과 생장

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히아신스 Pink Pearl 품종의 인편배양에서 자구의 재생과 생장은 1.0 mg/l와 3.0 mg/l의 농도에서 IAA 보다 IBA가 더 효과적이었으며, 절편체의 기부가 배지에 삽식되는 정상적인 치상 방향이 역방향의 치상 방법에 비해 자구의 재생과 뿌리의 생장에 더 효율적인 것으로 나타났다. 계대배양에서 재생된 자구와 뿌리의 생장은 한천배지보다 펄라이트가 첨가된 액체배지에서 증가되었다. 이와 같은 히아신스 Pink Pearl 품종의 기내배양의 경우 자구 재생을 위한 초대배양은 한천 고체배지를, 재생된 자구의 생장은 펄라이트의 액체배지를 사용하는 2 단계의 교대배양 시스템이 더 유용함을 시사한다.