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Systematic Propagation of High Quality Garlic (*Allium sativum* L.) Through Shoot Apical Meristem Culture III. Micropropagation by Involucre Culture

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생장점배양에 의한 우량 마늘의 체계적 증식 III. 총포배양에 의한 무병주 대량증식

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This study was conducted to obtain some basic information needed for the propagational system of high quality garlic through the culture of healthy tissues. When shoot-tips of bulbil obtained in mid May were cultured on MS medium containing 8% sucrose supplemented with 0.1 mg/L NAA, in vitro bulblets were formed, but the shoots were formed at the early to middle in June. Multiple shoots were induced by the culture of receptacles on MS medium supplemented with 0.1 mg/L NAA and 2 mg/L BA or 1 mg/L NAA and 10 mg/L BA. Among the flower bud, bulbil and receptacle, receptacle showed most suitable in terms of shoot formation efficiency. More than 50 shoots per single involucre were produced under the optimum condition. Results indicate that in vitro culture of involucre has a high potential for the micropropagation of high quality seed bulbs.

Key words: bulbil, flower bud, receptacle

If the complete stock of a cultivar is not infected, it should be possible to develop healthy basic stock through the selection of healthy plants and propagation vegetatively. However, when the entire population of the clone is infected, the only way to obtain pathogen-free stocks is to eradicate the pathogen from vegetative parts of the plants and to regenerate whole plants from such tissues. Garlic propagated by vegetative organs for several years are systematically infected with one or more pathogens, especially virus (Walkey et al., 1987). Virus-free garlic could be obtained by shoot-tip and callus cultures (Min et al., 1991; Lee and Lee, 1994). However, small shoot-tip explants have a low survival rate and show slow initial growth, and the frequency of shoot differentiation from callus culture decreases when it is subcultured for a long period (Choi et al., 1993). Once a single pathogen-free plant is obtained, it can be multiplied vegetatively by the formation of adventitious shoots under pathogen-free conditions.

This study was conducted to develop an effective mass propagation technique of high quality seed bulbs by the formation of shoot from the flower buds, bulbils or receptacles of involucre cultured under the aseptic condition.

MATERIALS AND METHODS

Culture of Bulbil Shoot-Tips

Involucres, flower heads, obtained from garlic plants grown in a net house were dipped into 98% of ethanol for 7 or 8 sec and flammed for sterilization. The shoot-tips of immature bulbil reached 0.5 to 1 mm in diameter were excised under a stereomicroscope and explanted from May 14 to June 14, 1990 at one- or two-day intervals. MS medium containing 8%

sucrose supplemented with 0.1 mg/L NAA was used. The media were solidified with 8 g/L agar and were autoclaved at 121°C for 15 min. The pH of the medium was adjusted to 5.8 with 1 N NaOH before autoclaving. The explants were cultured at 24 ± 3°C under the light conditions of 2,500 lux and 16h photoperiod. Bulb formation and shoot formation were observed after 18 weeks of culture.

Flower Bud, Immature Bulbil and Receptacle Cultures

The involucre spathe was removed and all visible flower buds, bulbils and receptacles were used as explants. Some receptacles were cut into radial quarters. MS medium supplemented with 0, 2, 5, or 10 mg/L BA and 0 or 1 mg/L NAA was used. Explants were cultured for 2 months since June 15, 1987. In second experiments carried out from June 4, 1991, explants were cultured on MS media supplemented with 2 mg/L BA, 0.1 mg/L NAA and 2 mg/L BA, or 1 mg/L NAA and 10 mg/L BA. The other conditions of medium and culture were the same as the previous experment.

RESULTS

Explanting Date of Shoot-Tips of Bulbil

To propagate high quality garlic in vitro, shoot-tips of bulbil were cultured on MS medium containing 8% sucrose supplemented with 0.1 mg/L NAA. The formation of shoot explanted on May 14 was 42.3%, but over 80% of explants were formed when those were cultured from May 28 to middle of June. Development of bulblet explanted on May 14 and 21 were about 40% but that decreased when those were explanted in early or middle of June. Thus, shoots were formed in the late explanting but in vitro bulblet developed in the early explanting (Figure 1).

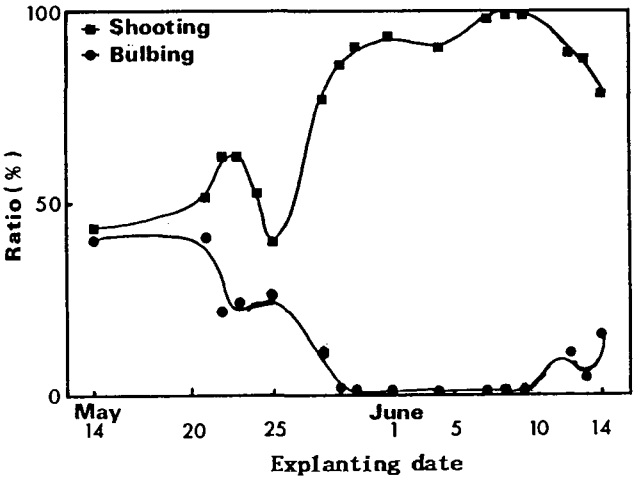

Figure 1. Comparison of ratio of shoot formation and bulbing from shoot-tip of bulbils explanted from May 14 to June 14, 1990.

Table 1. Effects of growth regulators on organ formation from flower bud, bulbil and receptacle of garlic cv Seosanjong cultured on June 15, 1987.

Explants	NAA (mg/L)	BA (mg/L)	Survival (%)	Shooting (%)	Callus (%)
Flower bud	0	0	80	0	0
	0	2	60	0	0
	1	2	90	0	0
	1	10	90	0	0
Bulbil	0	0	100	0	0
	0	2	90	78.6	0
	1	2	100	92.5	0
	1	10	80	94.4	0
Receptacle	0	0	70	0	0
	0	2	70	17.9	0
	1	2	60	12.5	37.5
	1	10	100	7.5	77.5

Propagation by Flower Bud, Immature Bulbil and Receptacle Cultures

The flower bud, bulbil and receptacle of garlic plant developed by shoot-tip culture in the previous experiment were cultured for 2 months from June 15 to August 15, 1987. The survival rate of explant was high in bulbil culture and the formation rate of shoot was 92.5% on MS medium supplemented with 1 mg/L NAA and 2 mg/L BA (Table 1). Whereas, when receptacles were used as an explant, the survival rate was low, and the shoot formation rate was 17.9% on MS medium with 2 mg/L BA and 12.5% on medium with 2 mg/L BA and 1 mg/L NAA. On the other hand, the shoots did not develop from flower bud under any treatment. Calli were induced on MS medium supplemented with the combination of 1 mg/L NAA and 2 or 10 mg/L BA in receptacle culture, but the calli were not formed on the other media or explants.

To investegate the effects of other explanting date, the organs of involucre were cultured on June 4, 1991 for 2 months (Table 2). When flower buds were cultured on

Table 2. Effects of growth regulators on organ formation in flower bud, bulbil and receptacle of garlic cv Seosanjong cultured on June 4, 1991.

Explants	NAA (mg/L)	BA (mg/L)	Survival (%)	No. shoots	Shoot height(mm)	Callus (%)	Fresh wt.(g)	Bulbing (%)
Flower bud	0	0	17.4	1.1	0.4	0	0.0	12.5
	0	2	85.7	0.5	2.6	88	0.1	0
	0.1	2	94.4	0.7	1.8	100	0.1	2.9
	1	10	95.5	0.5	1.6	100	0.2	0
Bulbil	0	0	81.8	1.2	14.0	0	0.4	3.7
	0	2	97.4	2.0	90.4	0	0.7	0
	0.1	2	92.6	2.5	53.5	0	0.7	0
	1	10	88.6	2.4	61.3	13	1.3	0
Receptacle	0	0	68.1	1.2	20.0	0	0.2	1.3
	0	2	71.4	4.3	71.9	0	0.5	0.1
	0.1	2	86.1	5.7	56.1	0	0.6	0
	1	10	73.3	5.8	49.9	13	1.0	0
					F value[a]			
Explant			-	39.4**	16.98**	107.39**	6.94**	NS
Growth regulator			-	4.03*	6.38**	17.86**	4.49**	3.33*
Explant × Growth regulator			-	2.84*	NS	12.28**	NS	NS

[a]NS, *, **: nonsignificant and significant at P=0.05 or P=0.01, respectively.

medium without growth regulators, the survival rate of flower buds was only 17.4%, but bulbil and receptacle was 81.8% and 68.1%, respectively. The survival rate of all explants reached 71.4 to 97.4% in the media with 0-1 mg/L NAA and 2-10 mg/L BA. The shoot formation frequency was relatively high in all kinds of explants. However, bulbils and receptacles produced higher numbers of shoots than flower buds. Especially, the receptacle culture on MS medium supplemented with 0.1 mg/L NAA and 2 mg/L BA or 1 mg/L NAA and 10 mg/L BA showed 5.7 or 5.8 shoots in average. The growth of shoot in bulbil and receptacle culture was higher than that in flower bud culture (Figure 2). The callus formation rate of bulbil and receptacle culture was low but that of flower bud cultured on medium supplemented with BA and NAA was 88 to 100%. The fresh weight of plantlet obtained from bulbil and receptacle MS medium supplemented with growth regulators were higher than those from flower buds. Bulblets were formed on the medium without growth regulators in all kinds of explants but the bulb growth was poor.

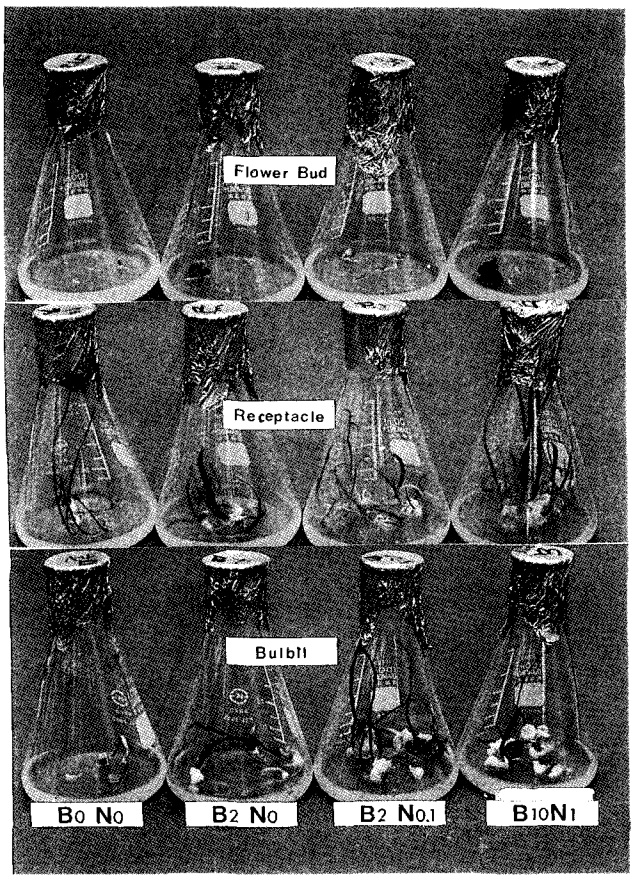

Figure 2. Organ differentiation from flower bud, receptacle and bulbil culture on MS medium supplemented with 0, 2 mg/L BA, 2 mg/L BA and 0.1 mg/L NAA, or 10 mg/L BA and 1 mg/L NAA (from left to right) cultured on June 4, 1991.

DISCUSSION

It is reportedly possible to produce virus-free stocks on a large scale by in vitro culture of vegetative and floral buds of garlic in a fast growing period (Yang et al., 1993). In this study, when shoot-tips of bulbils were cultured on MS medium containing 8% sucrose supplemented with 0.1 mg/L NAA, bulblets were formed at a frequency of about 40% on May 14 to 20. It is valuable to obtain bulblets directly from shoot-tips without shoot formation due to saveing time and labor.

It was difficult to obtain multiple shoots by the conventional method of shoot-tip culture of garlic. If a single pathogen-free plant is obtained, it can be multiplied vegetatively by secondary shoot induction as subculture of the plantlet. Dunstan and Short (1977, 1979), Matsubara and Hihara (1978) reported the induction of plantlet from in the culture of excised capitulum tissue of *A. cepa* on medium containing a high concentration of cytokinin. Novak and Havel (1981) observed the ability of plant regeneration on the in vitro cultured involucres of *A. cepa*, *A fistulosm* × *A.*

cepa, *A. sativum*, *A. flavum* and *A. porrum*., and Chio et al. (1992) did on flower stock of *A. sativum*. Reliable regeneration of adventitious shoots from explants in immature floral stems in tulips has been achieved by Alderson et al. (1983). It was also reported that more then 1,000 plantlets could be obtained from a single inflorescence of hyacinth in a period of 4 to 6 months (Kim et al., 1981). Bulblet formation of lilies was affected by several factors such as the time of excision and source of the explant. Bulbil development was quite fair in leaf explants excised before flowering, and mainly in those excised within 15 mm from the leaf base whereses, bulbils were seldom induced in explants excised after flowering regardless of their origin (Niimi and Onozawa, 1979). The regeneration of shoots from explants of immature flower bud of garlic was poor and 2.5 shoots was induced from the bulbil culture from early to middle in June. In the receptacle culture, shoot regeneration was stimulated on medium containing BA and NAA. The shoot regeneration ability of receptacle was more predominant than that of flower bud and bulbil under the optimum condition. This reason was thought that receptacle had many primodia of flower bud and buibil. Thus, we obtained over 50 shoots from a single involucre of garlic. It is considered that methods of plant production by involucre culture descrived useful for micropropagation of high quality seed bulb of garlic.

적 요

생장점배양하여 얻어진 소수의 식물체를 기내 재배양하여 건전 우량마늘을 대량으로 증식시킬 수 있었다. MS 기본배지에 서당 8%, NAA 0.1 mg/L 첨가된 배지에 지엽을 뚫고 나온 직후의 주아 생장점을 5월 중순에 치상하면 shoot의 형성 없이 기내인경이 직접 형성되어 배양 기간 및 노력을 절약할 수 있었다. 또한 총포 내의 미숙주아 및 receptacle을 6월 상순경에 0.1 mg/L NAA + 2 mg/L BA 혹은 1 mg/L NAA + 10 mg/L BA가 첨가된 MS 기본배지에 배양하면 multiple shoot가 유도되었다. 특히 여러 화기 재료 중 receptacle에서 평균 5.8개의 shoot가 분화되어 1개의 총포 내에서 50개 이상 분화되었다. 따라서 총포배양에 의한 식물체 생산은 우량마늘의 대량번식 체계의 한 수단으로 이용할 수 있었다.

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