STUDIES ON THE MORPHOGENESIS OF ASPARAGUS

IV. The effect of transplanting on callus and organ formation of stem segment cultured *in vitro*

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Introduction

In our previous papers[3,13,14], we described the effect of auxins and cytokinins on callus and organ formation from stem segments of asparagus seedling. These experiments were carried out using the same medium over a long term without transplanting.

It has been well known that the transplanting of the cultures from a medium containing auxins and cytokinins onto other media without growth regulators has a considerable effectiveness on organ formation. In the present experiment, the effects of transplanting in the early stages of culture on organ formation from stem segments of seedlings were investigated.

Materials and Methods

Seeds of asparagus (cv. Mary Washington 500) were surface-sterilized with 70% ethanol for 5 minutes and 10% antiformin (10% available chlorine) for 30 minutes, and washed with sterile distilled water. Then, these seeds were sown on medium which contained 20 g/l sucrose and 7 g/l agar and poured by 30 ml in 100 ml Erlenmyer flask, and were kept at 25°C in the drak for about 2 weeks.

When the first shoot of seedling elongated by 5–6 cm in length, approximately 1 cm-long stem segments were derived from the internode of middle portion of the first shoot and were placed on the medium containing MURASHIGE and SKOOG's organic and inorganic substances[9], 20 g/l sucrose, various concentrations of N^6-benzyladenine (BA) and NAA, and 7 g/l agar.

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TABLE 1. Concentrations of BA and NAA in each medium in pre-culture before transfer

Growth regulator	Mark of media							
	A	B	C	D	E	F	G	H
BA (mg/l)	0	0	0	0.1	1.0	1.0	1.0	10.0
NAA (mg/l)	0	0.1	1.0	1.0	0	1.0	10.0	1.0

The concentrations of BA and NAA in each treatment were shown in Table 1. pH of the media was adjusted to 5.5.

Three stem segments were placed onto the medium (poured by 25 ml per one 100 ml Erlenmyer flask) and 40 flasks (120 segments) were used in one treatment.

On the 3rd, 7th and 14th day after the beginning of pre-culture, 30 segments (10 flasks) of each treatment were transferred onto the media without growth regulators of which the constituent was similar to the media mentioned above except for containing no growth regulator.

The cultures were incubated under constant temperature condition (25°C) and light conditions were set in the dark during the first 4 weeks and then under 16-hour illumination per day with a 40-watt daylight fluorescent lamp (4,000 lx).

The size of the callus was indexed as follows; Index of callus size 0: no callus growth, 0.5: as large as a rice grain, 1.0: as large as an Azuki bean, 2.0: as large as a soybean, Index of more than 3 was based on the size of a soybean, for example, Index 3 is twice as large as a soybean, Index 4 is three fold larger than a soybean.

Results

1. Callus formation

Calluses were induced on the cross section of the segments, and then continued proliferation to form callus clumps. Callus formation was not observed in the media without NAA (medium A and E) regardless of transfer. In all other media containing 0.1–10.0 mg/l NAA, callus formation was observed within one or two weeks, and the percentage of segments inducing callus reached 90–100% within 4 weeks after the beginning of culture. In the case of long-term culture without transferring, good callus growth was obtained in media containing both BA and NAA (medium D, F, G and H) and callus growth was best in medium F containing 1.0 mg/l of BA and 1.0 mg/l of NAA (Fig. 1).

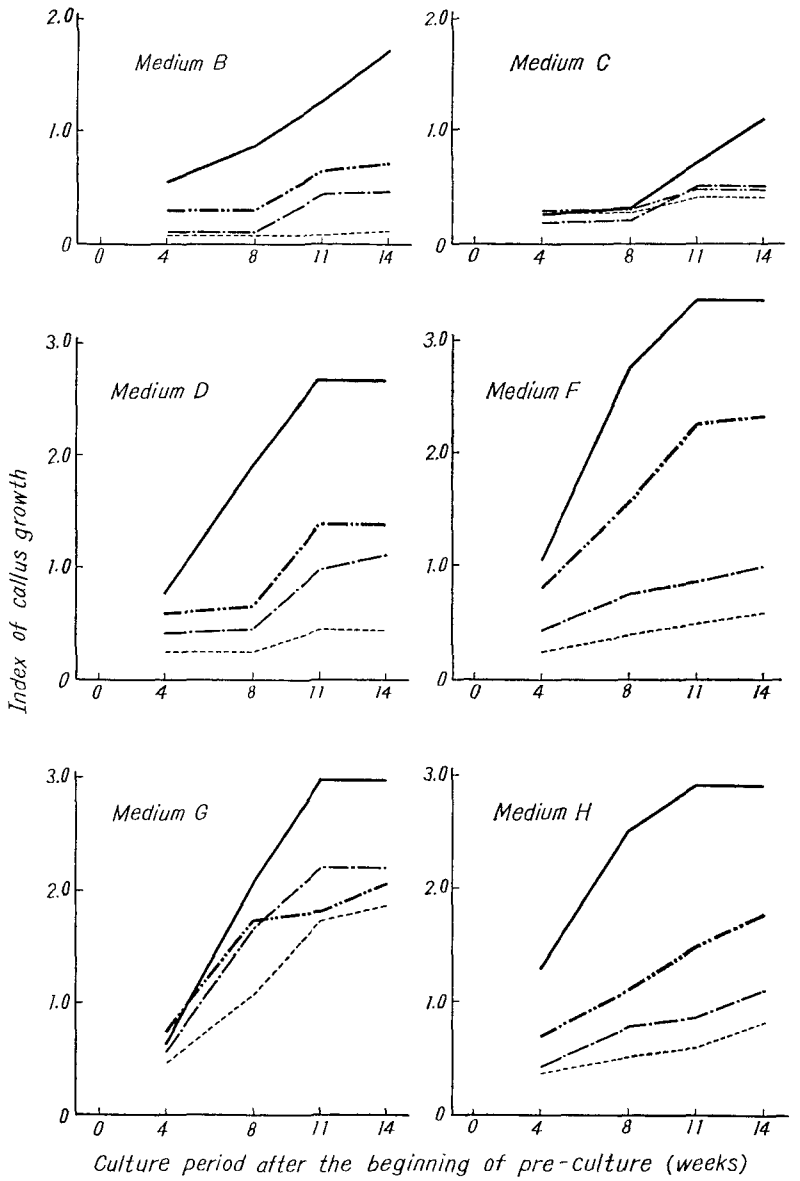

Fig. 1. Effect of growth regulators and transferring on callus growth.
——— long-term culture without transferring —·—·— 7-day pre-culture
— · · — 14-day pre-culture ····· 3-day pre-culture

In the case of a subculture, callus growth in transferring-batches was worse than that in a long-term culture without transferring regardless of the media constituent. Namely, calluses in the batches of 14-day preculture were 1/2–2/3 in the size of calluses in the batches of non-transferring, and callus growth in the batches of 3-day pre-culture was very bad in each medium except for medium G. In the batches of 7-day pre-culture, callus growth were between those of 3-day and 14-day pre-culture in each medium.

2. Root formation

All roots formed in this experiment redifferentiated from callus proliferat-

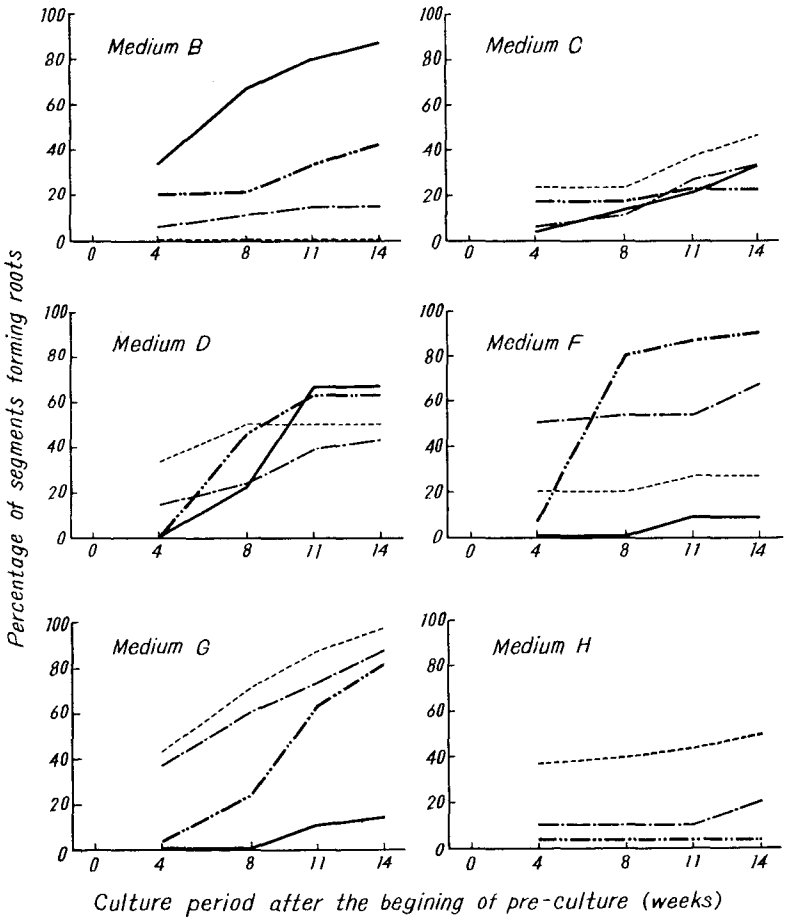

Fig. 2. Effect of growth regulators and transferring on root formation.
——— long-term culture without transferring —·—·— 7-day pre-culture
— · · — 14-day pre-culture ····· 3-day pre-culture

ing on segments. Root formation from callus was observed in all media in which callus was formed (medium B, C, D, F, G and H) and a high percentage of root formation was obtained in medium B, F and G (Fig. 2). In medium B in which none of BA and 0.1 mg/l of NAA were added, the percentage of segments forming roots was highest in the batch of a long-term culture without transferring and the percentage became lower with the transferring treatment, and 0% in the batches of 3-day pre-culture (Fig. 2-B). In addition, in the medium F, G and H in which high concentrations of BA and NAA were added, root formation was enhanced by transferring. Namely, in the medium F (1.0 mg/l BA and 1.0 mg/l NAA), root formation was best in the batches of 14-day pre-culture, and lowest in the batches of non-transferring (Fig. 2-F). In the medium G (1.0 mg/l BA and 10.0 mg/l NAA) and H (10.0 mg/l BA and 1.0 mg/l NAA), percentage of segments forming roots was highest in the batches of 3-day pre-culture and lowest in the batches of non-transferring (Fig. 2-G, H).

In the medium C and D, root formation from callus was observed in all batches but the effect of transferring on root formation was not recognized.

In all batches in this experiment, the percentage of segments forming roots was the highest (96.7%) in the batches of 3-day pre-culture on medium

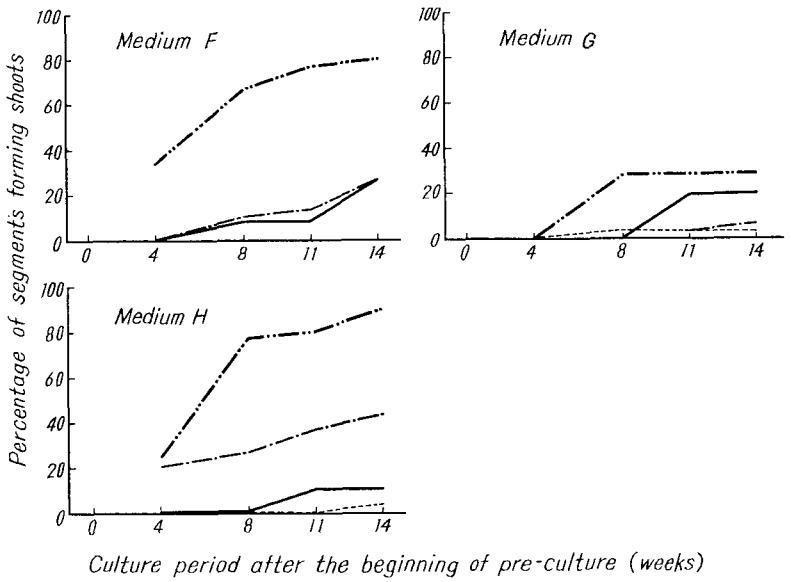

Fig. 3. Effect of growth regulators and transferring on shoot formation

——— long-term culture without transferring —·—·— 7-day pre-culture
— · · — 14-day pre-culture ····· 3-day pre-culture

G, followed by that (90.0%) in the batches of 14-day pre-culture on medium F, and third (86.7%) in the batches using medium B without transplanting.

3. Shoot formation

All shoots formed in this experiment redifferentiated from callus proliferating on segments. Shoot formation from callus was observed in the media (medium F, G and H) in which high concentrations of BA and NAA were added. In these cases, shoot formation was enhanced conspicuously by transferring to a medium without growth regulators. Namely, the percentage of shoot formation was about 80%-90% in the batches of 14-day pre-culture on medium F and H, and was 10-20% in the batches of non-transferring (Fig. 3). In medium G, shoot formation from the callus was observed in all batches, but the percentage of segments forming shoots was low (2-30%).

The percentage of segments forming both roots and shoots was highest in the batches of 14-day pre-culture on medium F (1.0 mg/l BA and 1.0 mg/l NAA).

Discussion

Generally, it has been well known that auxin and cytokinin is effective for callus and organ formation in the tissue culture of numerous kinds of plant[10,11,12,15,16,17]. In this experiment, callus formation was observed in all the media containing 0.1-10.0 mg/l NAA, but was not observed in the media without NAA. in the case of non-transferring treatment, a good callus growth was observed especially in a medium containing both BA and NAA, and callus growth was best at 1.0 mg/l BA and 1.0 mg/l NAA. These results are the same as that in the previous papers[3,5,13,14].

In each medium, callus growth was best in the batches of a long-term culture using the medium containing growth regulators without transferring, and callus growth became worse with transferring, especially in the case of a short-day pre-culture. From these results, it was clarified that long-term supplementing of growth regulators may be effective for callus growth and it was shown that the transferring in the early stage (3-14 days) is not effective.

Root formation was observed in all media containing 0.1-10.0 mg/l NAA, but the percentage of segments forming roots varied with concentrations of BA and NAA in the media used for pre-culture. Namely, in the case of long-term culture without transferring, the percentage of root formation was highest in medium B (0.1 mg/l NAA only), followed by that in medium D

(0.1 mg/l BA and 1.0 mg/l NAA) and was low in other media. This result is the same as that in the previous papers likewise[3,14].

For the purpose of differentiation of organs, explant is usually transferred onto the medium in which concentrations of growth regulators are different from that in the pre-culture[1,2,4,6,7,8,18]. In this experiment, effectiveness of transferring on root formation varied with the concentrations of BA and NAA in the media used for pre-culture. Namely, in medium B, the percentage of root formation became lower through transferring treatment, but in medium F (1.0 mg/l BA and 1.0 mg/l NAA), medium G (1.0 mg/l BA and 10.0 mg/l NAA) and medium H (10.0 mg/l BA and 1.0 mg/l NAA), root formation was enhanced conspicuously by transferring to medium without growth regulators. In these cases, the optimum term of pre-culture for root differentiation was also varied with concentrations of BA and NAA in the medium used in pre-culture. In other words, in medium F, root formation was best in 14-day pre-culture and in the medium H, the percentage of segments forming roots was highest in 3-day pre-culture. From these results, it could be said that when high concentrations of growth regulators was added to the medium of pre-culture, explants will absorb a sufficient amount for root formation within the short term of pre-culture.

Shoot formation was observed only in the media containing both BA and NAA. In these media, the percentage of shoot formation was low (less than 20%) in the long-term culture without transferring, and became higher through transferring, especially in the batches of 14-day pre-culture using medium F and H. From these results, for shoot formation, optimum concentration of growth regulators in pre-culture appears to be 1.0–10.0 mg/l BA and 1.0 mg/l NAA, and optimum term of pre-culture appears to be 14 days.

Summary

Stem segments 1.0 cm in length derived from the internode of the first stem of asparagus seedlings were cultured aseptically on MS medium to which various concentrations of N^6-benzyladenine (BA) and NAA were added. On the 3rd, 7th and 14th day after the beginning of culture, 30 segments in each medium were transferred onto the MS medium without growth regulators, and the effectiveness of transferring on callus and organ formation was recognized. The experimental results are summarized as follows:

1. Callus formation was observed in all media containing 0.1–10.0 mg/l NAA, but good callus growth was observed on a medium containing both BA and NAA. Effectiveness of transferring on callus growth was not

recognized. Namely, in each medium, callus growth was best in the batch of a longterm culture on medium containing growth regulators. In all media, callus growth was best at 1.0 mg/l BA and 1.0 mg/l NAA.

2. Root formation from callus was observed in all media in which callus were formed, but the percentage of root formation varied with concentrations of BA and NAA. The effectiveness of transferring on root formation also varied with concentrations of BA and NAA in media used in pre-culture. In all bactches, a high percentage of root formation was obtained in the batch of 3-day pre-culture on the medium G (1.0 mg/l BA and 10.0 mg/l NAA), 14-day pre-culture using the medium F (1.0 mg/l BA and 1.0 mg/l NAA) and continuous culture on the medium B (0.1 mg/l NAA only) without transferring.

3. Shoot formation from the callus was observed in the medium in which high concentrations of BA (1.0 or 10.0 mg/l) and NAA (1.0 mg/l) were added, and was enhanced conspicuously by transferring to the medium without growth regulators on the 14th day after the beginning of pre-culture.

In all batches in this experiment, the percentage of segments forming both roots and shoots was highest in the batch of 14-day pre-culture on medium F (1.0 mg/l BA and 1.0 mg/l NAA).

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