



HOKKAIDO UNIVERSITY

Title	Studies on the Morphogenesis of Asparagus : IV. The effect of transplanting on callus and organ formation of stem segment cultured in vitro
Author(s)	YAKUWA, Toshiro; HARADA, Takashi; TSUJI, Hozue
Citation	Journal of the Faculty of Agriculture, Hokkaido University, 61(1), 151-159
Issue Date	1982-11
Doc URL	https://hdl.handle.net/2115/12976
Type	departmental bulletin paper
File Information	61(1)_p151-159.pdf



STUDIES ON THE MORPHOGENESIS OF ASPARAGUS

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

Toshiro YAKUWA, Takashi HARADA
and Hozue TSUJI

Department of Horticulture, Faculty of Agriculture,
Hokkaido University, Sapporo 060 Japan

Received April 30, 1982

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 Erlenmeyer flask, and were kept at 25°C in the dark 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 substances⁹, 20 g/l sucrose, various concentrations of N⁶-benzyladenine (BA) and NAA, and 7 g/l agar.

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).

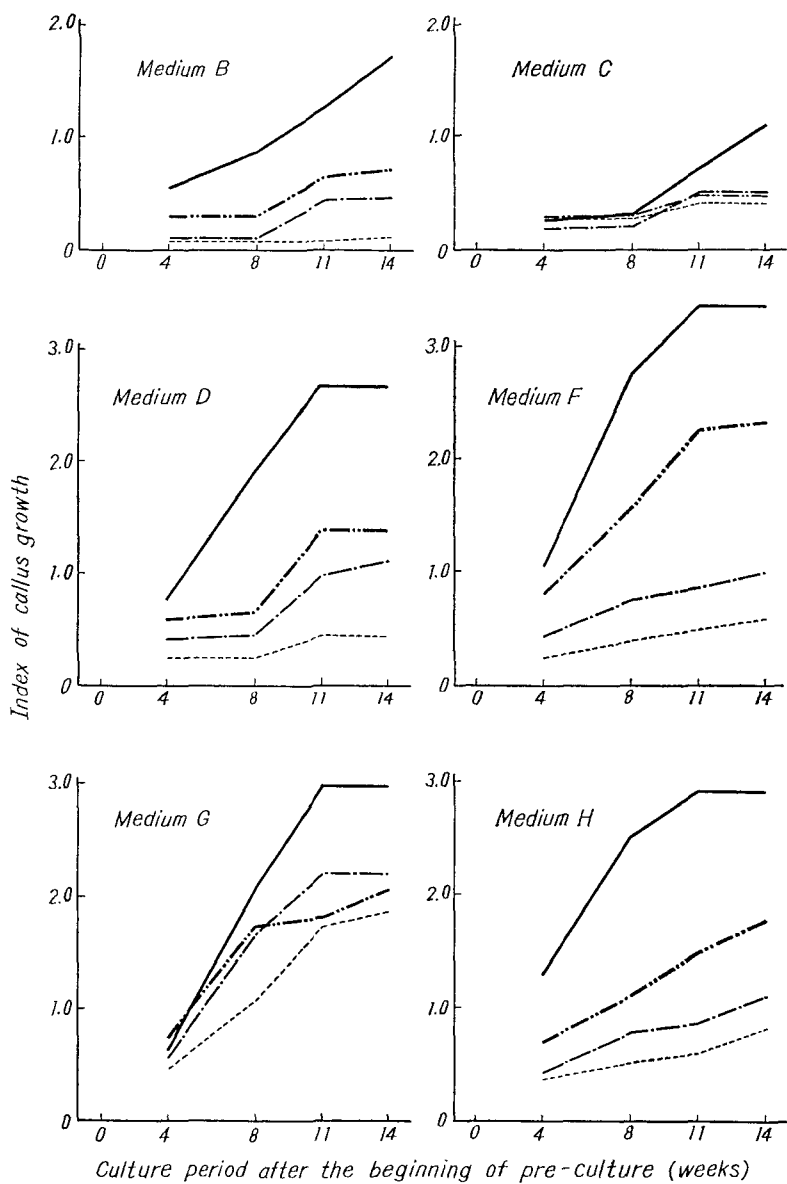


Fig. 1. Effect of growth regulators and transferring on callus growth.
 — long-term culture without transferring - · - · - 7-day pre-culture
 - · · - 14-day pre-culture · · · · · 3-day pre-culture

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-

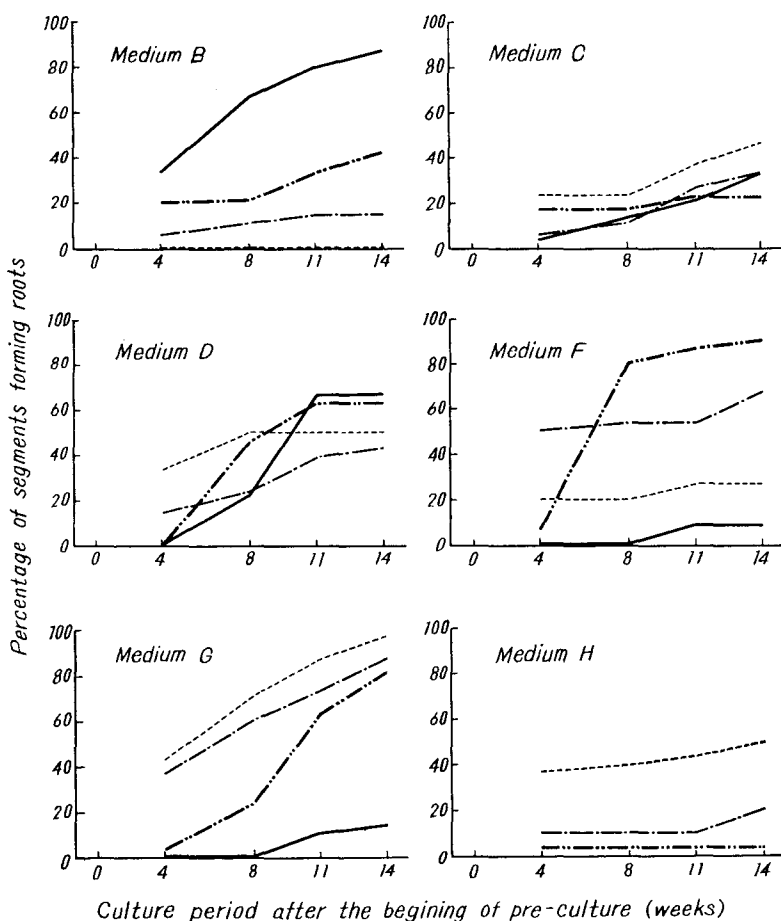


Fig. 2. Effect of growth regulators and transferring on root formation.

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

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 N⁶-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 batches, 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).

Literature Cited

1. DORÉ, C.: La multiplication clonale de l'asperge (*Asparagus officinalis* L.) par culture *in vitro*: son utilisation en sélection. *Ann. Amélior. Plantes*, **25**: 201-224. 1975
2. GREINER, H. D.: Vegetative Vermehrung von Spargel (*Asparagus officinalis* L.) durch die Kultur von Sproßspitzen. *Gartenbauwissenschaft*, **39**: 549-554. 1974
3. HARADA, T. and YAKUWA, T.: Studies on the morphogenesis of asparagus. III. A long-term observations on callus and organ formation in tissue culture (in Japanese). *Mem. Fac. Agr. Hokkaido Univ.*, **8**: 176-181. 1972
4. HASEGAWA, P. M., MURASHIGE, T. and TAKATORI, F. H.: Propagation of asparagus through shoot apex culture. II. Light and temperature requirements, transplantability of plants, and cytological characteristics. *J. Amer. Soc. Hort. Sci.*, **98**: 143-148. 1973
5. INAGAKI, N., HARADA, T. and YAKUWA, T.: Studies on the anther culture of horticultural crops. II. Callus formation from the anthers of asparagus (in Japanese). *J. Japan. Soc. Hort. Sci.*, **49**: 71-78. 1980
6. INAGAKI, N., HARADA, T. and YAKUWA, T.: Studies on the anther culture of horticultural crops. IV. Effect of growth regulators on organ formation from anther-derived callus of *Asparagus officinalis* L., *J. Fac. Agr. Hokkaido Univ.*, **60**: 236-249. 1981
7. INAGAKI, N., HARADA, T. and YAKUWA, T.: Studies on the anther culture of

- horticultural crops. VI. Regeneration of plantlets from shoots obtained through the anther culture of *Asparagus officinalis* L., *J. Fac. Agr. Hokkaido Univ.*, **60**: 275-283. 1982
8. IRITANI, M., HARADA, T. and YAKUWA, T.: Basic studies on the vegetative propagation of horticultural plants. III. Callus and organ formation in the *in vitro* culture of tissue segments derived from carrot plant (in Japanese). *Mem. Fac. Agr. Hokkaido Univ.*, **12**: 281-291. 1981
 9. MURASHIGE, T. and SKOOG, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiol. Plant.*, **15**: 473-497. 1962
 10. MURASHIGE, T., SHABDE, M. N., HASEGAWA, P. M., TAKATORI, F. H. and JONES, J. B.: Propagation of asparagus through shoot apex culture. I. Nutrient medium for formation of plantlets. *J. Amer. Soc. Hort. Sci.*, **97**: 158-161. 1972
 11. STEWARD, F. C. and MAPES, M. O.: Morphogenesis and plant propagation in aseptic cultures of asparagus. *Bot. Gaz.*, **132**: 70-79. 1971
 12. TAKATORI, F. H., MURASHIGE, T. and STILLMAN, J. I.: Vegetative propagation of asparagus through tissue culture. *Hortscience*, **3**: 20-22. 1968
 13. YAKUWA, T., HARADA, T., SAGA, K. and SHIGA, Y.: Studies on the morphogenesis of asparagus. I. Callus formation originating in the pith tissue of asparagus spears in tissue culture (in Japanese). *J. Japan. Soc. Hort. Sci.*, **40**: 230-236. 1971
 14. YAKUWA, T., HARADA, T., SAGA, K. and SHIGA, Y.: Studies on the morphogenesis of asparagus. II. Effects of auxins and 6-benzyladenine on callus and organ formation of stem pieces cultured *in vitro* (in Japanese). *J. Japan. Soc. Hort. Sci.*, **40**: 347-353. 1971
 15. YAKUWA, T., HARADA, T., INAGAKI, N. and SHIGA, Y.: Studies on the anther culture of horticultural crops. I. Callus and organ formation in anther culture of asparagus (in Japanese). *J. Japan. Soc. Hort. Sci.*, **41**: 272-280. 1972
 16. YAKUWA, T., HARADA, T. and KUROSAKI, T.: Basic studies on the vegetative propagation of horticultural plants. I. Callus and root formation from tissue segments of grape shoots in tissue culture (in Japanese). *Mem. Fac. Agr. Hokkaido Univ.*, **8**: 397-403. 1973
 17. YAKUWA, T., HARADA, T. and HANZAWA, M.: Basic studies on the vegetative propagation of horticultural plants. II. Effect of growth regulators on callus and organ formation from tissue segments of tomato cultured *in vitro* (in Japanese). *Mem. Fac. Agr. Hokkaido Univ.*, **9**: 25-41. 1973
 18. YANG, H. J. and CLORE, W. J.: Development of complete plantlets from moderately vigorous asparagus shoots of stock plants *in vitro*. *Hortscience*, **9**: 138-140. 1974