STUDIES ON THE MORPHOGENESIS OF ASPARAGUS

VII. Callus and organ formation in the *in vitro* culture of cladophylls

Takashi HARADA and Toshiro YAKUWA

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

Receivcd June 8, 1983

Introduction

Asparagus plants obtained through seed propagation and grown commercially show a fair range of variations of quantity and quality in productivity. The establishment of an efficient asexual reproduction method to obtain numerous plants of the same excellent characteristics is highly desirable to increase the productivity in asparagus growing.

For this purpose, some kinds of tissue cultures have been widely carried out using various kind of tissues of the asparagus plants.[1,2,3,4,5,8,9,10] In these cases, the materials used were an apical meristem, a shoot tip, a small, unbranched lateral shoot excised from young spears, an undeveloped lateral bud of spears and a pith tissues of spears. However, these materials are limited in number in the case of obtaining samples from only one plant, and are insufficient for use in mass propagation available for actual commercial cultivation of asparagus requiring numerous plants of particularly excellent characteristics.

To solve these problems, the authors pay attention to cladophylls of asparagus as one of the readily-obtainable materials, and carried out the *in vitro* culture of the cladophylls, which were developing in numbers of tens or hundreds of thousand from only a single plant.

Materials and Methods

Usually, cladophylls developing on the mature asparagus plants have differences in thickness, color and stiffness. In this experiment, three kinds of cladophylls were derived from 3 different mature plants which are 4-year old and belong to *Asparagus officinalis* L. cv. Mary Washington 500. The

first is the thickest, deepest of green and stiffest (represented by A in this experiment). The second is of a medium degree in the three characters (B), and the third is lowest of all (C).

Five-centimeter-length pieces of branched lateral shoots with cladophylls were surface-sterilized for 10 min. with sodium hypochloride solution containing 1% of active chlorine and a few drops of Tween 20.

The cladophylls were cut at a portion approx. 3 mm apart from a basal end, and three pieces of those were placed horizontally on 25 ml of solid media poured into a 100-ml Erlenmeyer flask.

The media used contained MURASHIGE and SKOOG's inorganic and organic substances, 0.1 M sucrose, 0.6% agar and growth regulators. The growth regulators were α-naphtaleneacetic acid (10^{-6} M and 10^{-5} M), β-indolebuthylic acid (10^{-6} M and 10^{-5} M) and N^6-benzyladenine (10^{-6} M), and were added to the media separately or in combination as shown in Table 1. Initial pH of the media was adjusted to 5.5. Also, the media were sterilized by autoclaving at 120°C for 15 minutes. The cultures were maintained under conditions of 25°C with 16-hour illumination per day (1,500 lx with a white fluorecent lamp).

Results and Discussion

The results after 18 weeks of culture are shown in Table 1. Callus induction began to be observed at the cut surface of the cladophylls after seven to ten days of culture. No callus was formed in the media containing no growth regulator such as auxins and cytokinins. In the case of an addition of auxins (10^{-6} and 10^{-5} M of NAA or IBA) alone, the callus induction was hardly observed or not recognized at all with both NAA and IBA. In contrast, it was seen in a fairly high percentage of 51 to 90% in a combined addition of auxins (10^{-5} M NAA or 10^{-5} M IBA) and cytokinin (10^{-6} M BA). The growth of callus was better in NAA than in IBA, and the size of the callus clumps was larger at 10^{-5} M of NAA combined with 10^{-6} M of BA than 10^{-5} M of IBA combined with 10^{-6} M of BA.

Callus induction was slightly observed in the media with no growth regulator in the previous experiments[7] in which the pith tissues excised from the spears and the shoot segments of the seedlings were cultured *in vitro*. On the contrary, in this experiment, no callus formation was seen in the media without either auxins and cytokinins, and was recognized in the case devoid of BA in the media, with a few exceptions. The differences between the two cases mentioned above are considered to result from whether growth regulators such as auxins and cytokinins are contained endogenously in the

TABLE 1. Callus and organ formation in the *in vitro* culture of asparagus cladophylls after 18 weeks of culture

Growth regulators (M)			Types of cladophylls[1]	Number of cultured segments	Callus formation			Root differentiation		Shoot differentiation	
NAA	IBA	BA			Number of segments	Percentage	Size of callus[2]	Number of segments	Percentage	Number of segments	Percentage
0	0	0	A	33	0	0		0	0	0	0
0	0	0	B	33	0	0		0	0	0	0
0	0	0	C	33	0	0		0	0	0	0
10^{-6}	0	0	A	30	0	0		0	0	0	0
10^{-6}	0	0	B	30	1	3.3	15	1	3.3	0	0
10^{-6}	0	0	C	30	0	0		0	0	0	0
10^{-5}	0	0	A	30	1	3.3	20	1	3.3	0	0
10^{-5}	0	0	B	30	0	0		0	0	0	0
10^{-5}	0	0	C	30	0	0		0	0	0	0
10^{-5}	0	10^{-6}	A	30	22	73.3	13	18	60.0	1	3.3
10^{-5}	0	10^{-6}	B	33	25	75.7	17	25	75.7	0	0
10^{-6}	0	10^{-6}	C	27	14	51.8	20	14	51.8	4	14.8
0	10^{-6}	0	A	30	0	0		0	0	0	0
0	10^{-6}	0	B	30	0	0		0	0	0	0
0	10^{-6}	0	C	30	0	0		0	0	0	0
0	10^{-5}	0	A	30	0	0		0	0	0	0
0	10^{-5}	0	B	30	1	3.3	10	1	3.3	0	0
0	10^{-5}	0	C	30	0	0		0	0	0	0
0	10^{-5}	10^{-6}	A	30	24	80.0	8	14	46.6	1	3.3
0	10^{-5}	10^{-6}	B	30	27	90.0	9	20	66.6	2	6.6
0	10^{-5}	10^{-6}	C	33	30	90.9	10	8	24.2	0	0

1) A : Cladophylls in the highest degree of the thickness, depth of green and stiffness.
B : Cladophylls in the medium degree of the characters.
C : Cladophylls in the lowest degree of the characters.

2) Figures show the diameter in millimeter of a callus clumps regarded as a sphere.

tissues cultured. Considering that cytokinins as well as auxins play an important role in the callus induction in the tissue culture of asparagus as reported in the previous paper[6], and that cladophyll pieces used in this experiment are very small (1 mm or less in thickness and 1～2 cm in length), the fact that no callus induction has been observed may be due to that the cladophylls endogenously have only a very slight amount of auxins and cytokinins. Two types of calli were formed: one was green, compact and proliferated not so rapidly, and the other was nongreen, friable and proliferated more rapidly and vigorously. The latter appeared to form shoots or roots more frequently.

Root differentiation from the calli was seen after six weeks of culture. The percentage of root-forming segments were approximately 51 to 75% in 10^{-5} M NAA combined with 10^{-6} M BA, while they were 24 to 66% at 10^{-5} M IBA combined with 10^{-6} M BA. The percentage was somewhat higher with the addition of NAA than with that of IBA. It was recognized that number of roots per segment was large with an addition of NAA and BA in combination, while it was small with that of IBA and BA in combination. In contrast, regarding the length of the roots, the ratio of short roots against all the roots in the batch was somewhat high in the medium containing both NAA and BA, and low with both IBA and BA, while the ratio of comparatively long roots was high with the addition of both IBA and BA, and was low with that of both NAA and BA. In other words, in the case of using NAA as auxins, short and semitransparent roots were formed on the surface of the callus clumps, but when IBA was used, the long and opaque roots, which were akin to a normal root with a physiological function as in that of seedlings, were obtained in higher frequency.

The shoots differentiated from the calli were observed only in the media with auxins (10^{-5} M NAA or 10^{-5} M IBA) combined with cytokinin (10^{-6} M BA) and some of the shoots grew to become seven centimeter in length. The percentage of the segments forming shoots and the number of the shoots was not so large (one to four per segments), but those were expected to increase with a further increase of culture period.

As far as the authors know, the cladophylls of asparagus prepared by the procedure as described in "Materials and Method" were cultured *in vitro* as an easily-obtainable material available for vegetative propagation for the first time in this experiment. The cladophylls developing in asparagus intact plants are akin to an aciculate leaf, whereas they are not leaves morphologically speaking, but a partial portion of a branch. In the previous experiment[3,5,8,9,10], in which various kinds of tissues of asparagus such as a

shoot pith or the shoot segments of seedlings were cultured, the approximate concentrations of 10^{-6} to 10^{-5} M of auxins and 10^{-6} M of cytokinins were frequently used for callus formation and organ differentiation. In the present experiment, 10^{-6} and 10^{-5} M NAA was used separately or in combination with 10^{-6} M BA, and, in addition, 10^{-6} and 10^{-5} M IBA also was added to the media separately or in combination with 10^{-6} M BA.

In the present experiment, three kinds of cladophylls derived respectively from three different plants were cultured, and all kinds of cladophylls formed calli from which roots and shoots differentiated. However, no clear relationship between the kinds of the cladophylls and the callus and organ formation was recognized. It is desirable that further investigations on the influence of the kinds of the cladophylls will be carried out on the basis of the individuality or characteristics of plants and the developmental stage of lateral shoots.

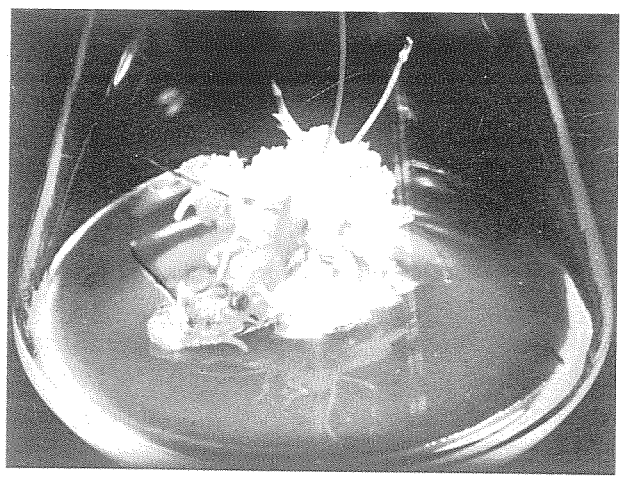

Fig. 1. Shoot and root formation from callus in the *in vitro* culture of asparagus cladophylls.

Consequently, the fact that callus and organ formation is observed in the *in vitro* culture of cladophylls of asparagus may suggest a capability of establishing a mass propagation method available for commercial cultivation in the future.

Summary

Cladophylls of asparagus (*Asparagus officinalis* L. cv. Mary Washington 500) were cultured *in vitro* as numerously-obtainable materials to establish

a mass propagation method available for commercial cultivation.

Three kinds of cladophylls, which were derived from three different plants and were in different degrees of thickness, green color and stiffness, were cut at the basal portion, and were cultured on the solid media containing MURASHIGE and SKOOG's prescription, 0.1 M sucrose, 0.6% agar and growth regulators (10^{-6} and 10^{-5} M NAA, 10^{-6} and 10^{-5} M IBA used separately or in combination with 10^{-6} M BA) under 25°C and 16-hour dayly illumination (4,000 lx).

Callus formation was observed in the media with both 10^{-5} M NAA and 10^{-6} M BA or both 10^{-5} M IBA and 10^{-6} M BA (51-90% in the ratio of callus-forming segments), while it was not recognized with NAA or IBA alone and none of the growth regulators, with a few exceptions.

Root differentiation from the calli was observed in the media containing both 10^{-5} M NAA and 10^{-6} M BA or both 10^{-5} M IBA and 10^{-6} M BA. Many short roots were formed with both 10^{-5} M NAA and 10^{-6} M BA, while comparatively longer roots were formed with both 10^{-5} M IBA and 10^{-6} M BA. Shoots slightly differentiated in the media with 10^{-5} M NAA combined with 10^{-6} M BA and 10^{-5} M IBA combined with 10^{-5} M BA. No clear difference in callus and organ formation was recognized between the three kinds of cladophylls used.

The fact that callus and organ formation are induced even slightly may suggests a possibility of establishing a mass propagation method available for the commercial cultivation of asparagus.

Literature Cited

1. ANDRESSEN, D. C. and ELLISON, J. H.: Root initiation of stem tip cuttings from mature asparagus plants, *Proc. Amer. Soc. hort. sci.* **90**. 158-162. 1967
2. FONNESBECH, M., FONNESBECH, A. and BREDMOSE, N.: Development of asparagus plumosus shoot tips grown *in vitro*, *Physiol. Plant.*, **40**: 73-76. 1974
3. GREINER, H. D.: Vermehrung von spargel (*Asparagus officinalis* L.) durch die kultur von sproßspitzen, *Gartenbauwissenshaft*, **39**: 549-554. 1974
4. MATSUBARA, S. and CLORE, W. J.: Vegetative propagation of asparagus from lateral buds, *Sci. Rep. Fac. Agric. Okayama Univ.*, **43**: 19-26. 1974
5. MURASHIGE, T., SHABDE, M. N., HASEGAWA, P. M., TAKATORI, F. H. and JONES, J. B.: Propagation of asparagus through apex culture, I. Nutrient medium for formation of plantlets, *J. Amer. Soc. Hort. Sci.*, **97**: 158-161. 1972
6. 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, *J. Jap. Soc. Hort. Sci.*, **40**: 230-236. 1971
7. YAKUWA, T., HARADA, T., SAGA, K. and SHIGA, Y.: Studies on the morpho-

genesis of asparagus, II. Effect of auxins and 6-benzyladenine on callus and organ formation of stem pieces cultured *in vitro*, *J. Jap. Soc. Hort. Sci.*, **40**: 347–353. 1971

8. YANG, Hsu-Jen and CLORE, W. J.: Rapid vegetative propagation of asparagus through lateral bud culture, *Hort Science*, **8**: 141–142. 1973
9. YANG, Hsu-Jen and CLORE, W. J.: *In vitro* reproductiveness of asparagus stem segments with branch shoots at a node, *HortScience*, **10**: 411–412. 1975
10. YANG, Hsu-Jen and CLORE, W. J.: Obtaining virus-free plants of *Asparagus officinalis* L. by culturing shoot tips and apical meristems, *HortScience*, **11**: 474–475. 1976