BASIC STUDIES ON THE VEGETATIVE PROPAGATION OF HORTICULTURAL PLANTS

IV. Callus formation in the *in vitro* culture of current shoot segments of highbush blueberry

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Introduction

Highbush blueberry (*Vaccinium corymbosum* L.) is one of the horticultural crops fairly difficult in vegetative propagation. In hard or soft wood cutting of highbush blueberry, various phenomena are observed: some roots emerge from the interior of the cuttings, callus is formed on the cut surface of the exterior of the bark, and in some cases, roots differentiate from the callus. In the first case, it is reported that a hard tissue in the cortex of branches of blueberry plays certain important roles in the difficult emergence of roots in hard wood cutting.[1] In addition, in the second and third cases, callus formation and root differentiation are of lower frequency according to the conditions provided.

The present study was designed to obtain a fundamental knowledge on callus and organ formation in the *in vitro* culture of blueberry tissues and to find out the adaptability of the application of tissue culture method to vegetative propagation of blueberry.

Materials and Methods

Tissues cultured were derived from current shoots of the 15-year-old plants of highbush blueberry (*Vaccinium corymbosum* L. cv. Burlington, growing in the experimental farm of Hokkaido University) on June 30. Soft wood segments 5 to 7 cm long, which were excised from the portion near a tip of the current shoots and from which a tip and leaves were removed, were surface-sterilized for 10 min. with sodium hypochloride solution containing 1% active chlorine and a few drops of Tween 20. Then, disks 1 mm in thickness were excised aseptically from the internode of the segments, and

were cultured on a solid meidum (described below) under the conditions of 25°C and darkness.

The number of the disk-shaped tissues cultured were 5 per 100-ml Erlenmeyer flask and 25 per batch.

The media used contained MURASHIGE and SKOOG's inorganic and organic substances, 2% sucrose, growth regulators, 0.7% agar. The growth regulators such as 0.01, 0.1, 1.0 and 10.0 mg/l of NAA and 0.1 and 1.0 mg/l of BA were used separately or in combination. The pH of the media was adjusted to 5.0 with NaOH or HCl before autoclaving. These media were pipeted by 25 ml into one Erlenmeyer flask, and were autoclaved at 120°C for 15 min.

Results and Discussion

Callus initiation began to be observed after 7 to 10 days of culture. Fig. 1 shows the percentage of segments forming callus.

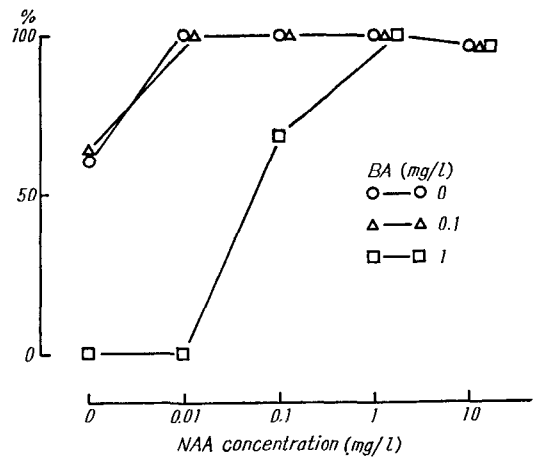

Fig. 1. Percentage of disk-shaped segments forming callus in the *in vitro* culture of current shoot tissues of highbush blueberry after 10 weeks of culture.

It was about 65% in the medium containing no growth regulator, and very high in the media containing NAA alone: 100% at 0.01, 0.1 and 1.0 mg/l of NAA, and above 95% at 10.0 mg/l of it. In the case of the combined addition of 0.1 mg/l of BA with 4 different concentrations of NAA, a similar tendency to that in using NAA alone was recognized in the percentages. However, in the case of adding 1.0 mg/l of BA, the percentage was 0 at

none or 0.01 mg/l of NAA, and fairly lower at 0.1 mg/l of NAA than that at the same concentration of NAA with none or 0.1 mg/l of BA. It was the same at 1.0 or 10.0 mg/l of NAA, regardless of the presence or absence of BA. From a viewpoint of balance in concentrations of both NAA and BA, BA showed an inhibitory effect on the incidence of callus initiation according to the combinations in which BA was equal to or higher than NAA in concentrations. In the case of 1.0 mg/l of BA, callus initiation was inhibited at NAA concentrations lower than BA concentrations, while the inhibitory effect of BA disappeared with NAA concentrations higher than BA concentrations. Generally in this experiment, an addition of BA gave no increase on the percentage of callus-forming tissues.

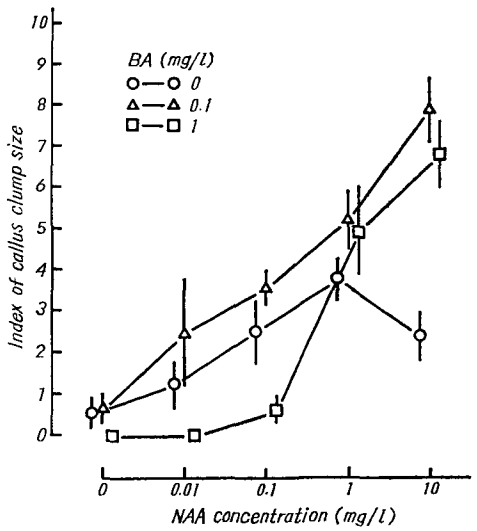

Fig. 2. Callus growth in the *in vitro* culture of current shoot tissues of highbush blueberry after 10 weeks of culture. Indices are represented as follows: Index 1 is equivalent to a size as large as the volume of a sphere 3 mm in diameter, index 2 equivalent to that of 5 mm, '3' to 7 mm, '4' to 9 mm, '5' to 11 mm, '6' to 13 mm, '7' to 15 mm and '8' to 17 mm, respectively. Vertical bars indicate SD.

Callus growth after 10 weeks of culture is shown in Fig. 2. A combined addition of 10 mg/l of NAA and 0.1 mg/l of BA gave the best callus proliferation. In the case of a separate addition of NAA, a curve of callus growth showed a peak at 1.0 mg/l, while at 10 mg/l, it declined considerably. As to the effect of BA, 0.1 mg/l of BA enhanced the callus growth in all combined additions with each of 4 different concentrations of NAA. Thus,

the callus growth was enhanced by the optimal addition of BA, and was different from the callus initiation. However, 1.0 mg/l of BA showed an inhibitory effect on the callus growth in the combined addition with none, 0.01 and 0.1 mg/l of NAA, while it gave a stimulatory effect with 1.0 and 10.0 mg/l of NAA. Especially paying attention to the callus growth at 10 mg/l of NAA, the followings are considered: calli grow vigorously even at high concentrations of NAA, if relatively optimal additions of BA are made, namely the effect of the excessive concentration of NAA is eliminated by suitable addition of BA.

LYRENE[2] reported that 0.25 to 0.50 mg/l of 2, 4-D added separately produced abundant callus growth in callus culture derived from stem segments of highbush blueberry, and also, NICKERSON and HALL[4] described that 0.1 or 0.5 mg/l of 2, 4-D added separately was most effective on the callus formation from stem internode sections of lowbush blueberry. In this experiment, although NAA was used as an auxin in place of 2, 4-D, it's optimal concentration is assumed to be approximately at 1.0 mg/l.

On the other hand, it was of considerable interest that in this experiment, the callus was initiated, although slightly, on a medium without growth regulators such as auxins and cytokinins. This seems to suggest that the tissues derived from current shoots contains some amounts of plant hormones affecting dedifferentiation. In addition, as described above, BA showed no enhancement in the callus initiation. This fact also indicates that some kinds of cytokinins exist endogenously in the current shoot tissue of blueberry.

In the present experiment, calli with various characteristics were initiated and proliferated according to the concentrations of both NAA and BA (Fig. 3). Some observed corelations between the characteristics of calli and the growth regulators added to media could be described as follows: white and powdery calli were observed at 0.1 or 1.0 mg/l of NAA alone; the compact calli with both a slight proliferation and a smooth and glossed surface were obtained at 10 mg/l of NAA alone; friable calli with a vigorous proliferation were initiated under the combined addition of optimal concentrations of both NAA and BA; in addition, fairly friable calli proliferating horizontally and widely along the medium surface were seen at a high concentration (10 mg/l) of NAA combined with BA. NICKERSON and HALL[4] observed that there were differences in the amount and friability of callus on intermediate sections taken from different clones. It is noteworthy in connection with organ differentiation in a successive subculture that in the present study variously characterized calli can be initiated according to the kinds and concentrations of the growth regulators added to the media.

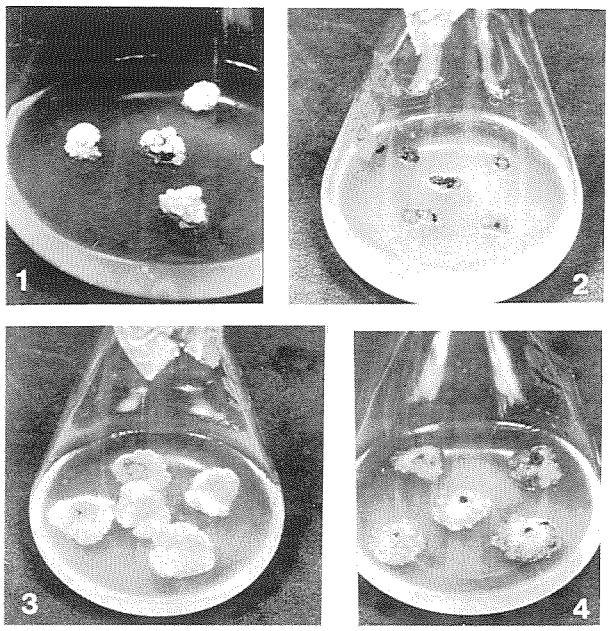

Fig. 3. Various characteristics of calli initiated from current shoot tissues of highbush blueberry (*Vaccinium corymbosum* L. cv. Burlington).
1: White and powdery calli.
2: Compact calli with the slight proliferation and the smooth, glossy surface.
3: Friable calli proliferating vigorously.
4: Fairly friable calli proliferating horizontally and widely along the medium surface.

In this study, all the cultures were maintained in the dark, based on the results reported by NICKERSON[4] in which callus formation was generally better in the dark than under light in the *in vitro* culture of stem internode sections of lowbush blueberry. However, it might be necessary to examine the light conditions in relation especially to the characteristics of the calli initiated. In the present experiment, none of both root and shoot differentiation from the calli are observed, but the authors are attempting to carry out further investigations on organ differentiation from these calli.

To date, various tissue cultures have been carried out to determine the capability of regeneration of plantlets of the blueberry. In one of those cases, root differentiation from the callus has been observed, whereas no shoot differentiation from callus has been obtained[6]. It is now possible to initiate roots in tissue culture of blueberry as reported by LYRENE[3] or NICKERSON[5] that some kinds of explants with a juvenility-juvenile shoots

severed from tissue culture colonies initiated from nonjuvenile explants in LYRENE's case, and cotyledons or hypocotyl sections exiced from seedlings in NIKERSON's case.

On the other hand, it seems to be desirable from the viewpoint of not only establishing a propagation method but also clarifying the morphogenesis of the callus originated from an arbor plant tissue that organogenesis of the callus derived from various tissues of the blueberry plant as a small fruit tree are investigated successively.

Summary

Disk-shaped tissues 1 mm in thickness, which were derived from the internode of current shoots of 15-year-old highbush blueberry plants (*Vaccinium corymbosum* L. cv. Burlington), were aseptically cultured *in vitro* on medium containing MURASHIGE and SKOOG's prescription, 2% sucrose, growth regulators —0, 0.01, 0.1, 1.0 and 10.0 mg/l of NAA and 0, 0.1, 1.0 mg/l of BA added separately or in combination —, 0.7% agar under the conditions of 25°C and darkness.

1. Callus initiation was observed after 7 to 10 days of culture. Percentage of callus initiation was 100% at 0.01, 0.1 and 1.0 mg/l, and 95% at 10.0 mg/l in using NAA alone. An addition of BA showed no enhancement on the percentage of callus-forming tissues.

2. Callus growth was best at 1.0 mg/l of NAA in the case of separate addition. An addition of 0.1 mg/l of BA in combination with NAA enhanced callus growth and also eliminated the reduction of callus growth by the excessive addition of NAA.

3. Variously characterized calli were obtained: the white and powdery calli were observed at 0.1 or 1.0 mg/l of NAA alone, the compact calli with a slight proliferation and a smooth, glossy surface were seen at 10.0 mg/l of NAA alone, the friable calli proliferating vigorously were obtained under the combined addition of optimal concentrations of both NAA and BA.

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