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Vegetative Propagation of Asparagus from Lateral Buds

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Introduction

Staminate asparagus plants produce higher yields than pistillate plants as they emerge earlier and usually produce more spears by number and weight. Asparagus is generally propagated by seed and the staminate and pistillate plants produced occur in equal numbers. Prior to blooming, the sex of a plant has been impossible to determine. Because of the heterogeneous character of this plant the tissue culture technique makes possible the propagation of genetically similar plants in large quantities. This offers some interesting possibilities both for research and commercial production.

Gorter[2], Takatori[5] and Wilmar[7] succeeded in the vegetative propagation of asparagus through the callus culture technique. All plantlets that Takatori obtained by this technique were the diploid chromosome complement. Malnassy et al.[3] gained asparagus tetraploids from the callus tissue culture by the method of Takatori et al.[6] and diploids from the rooted cutting by the method of Andreassen et al.[1]. We obtained some polyploids among diploidsf rom diploid crown through callus tissue culture. Takatori et al.[5] have succeeded also by using other methods such as lateral bud tissue culture and shoot apex culture (private communication). These methods induce only shoot and root growth, without callus formation, thus resulting in genetically similar and diploid plants from the diploid crown. We tried lateral bud culture by the methods described hereafter and obtained the following results.

Methods and Procedures

Procedure 1 :

Growth of several shoots from a lateral bud of spear was obtained, but no roots develop. This material can be used to supply additional material for sub-culturing.

Procedure 2 :

Several shoot and root growths were obtained from a node cut from a shoot in the first procedure.

Procedure 3 :

Plantlets from the second procedure were grown to transplantable size.

Murashige and Skoog's medium[4] containing 30 g/l sucrose and 7 g/l bacto agar was used as the basic medium through this experiment, unless otherwise noted. Before autoclaving the pH of the medium was adjusted to 5.7 by 1 N NaOH. These cultures were supplied with 0, 8, 16 and 24 hours of artificial light at 26°C. Good growth occurred under 16 and 24 hours of daylength supplied with 100 ft-c light from 20 watt

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Gro-Lux fluorescent lamps. The results under 16 hours daylength are reported in this experiment.

Procedure 1 :

A thirteen-year-old selected No. 11 male plant of *Asparagus officinalis* cultivar UC 500-W of a field planting was used. Spears emerging during the middle of April to the beginning of July, which were about 15 cm tall, were utilized as a source of explants. Two cm of the apex was discarded and the subjacent 7 cm section of each spear was used. The outermost scale of the lateral bud was first discarded and the surface was sterilized by washing twice with 10% commercial clorox (0.525% sodium hypochlorite) and immersing for 5 seconds in 70% ethanol and successively in 10% clorox solution for 15 minutes. The disinfected section was rinsed twice with sterilized water, then a bud with outer scales was excised for placing on the 50 ml medium in a 125 ml flask. Effects of NAA and kinetin on shoot growth were tested singly or in combination in concentration of 0, 12^{-2}, 10^{-1} or 1.0 mg/l of each chemical. Zero, 10^{-2} and 10^{-1} mg/l of NAA were the most effective on shoot growth. Each chemical of high concentration inhibited shoot growth, but caused callus or root formation.

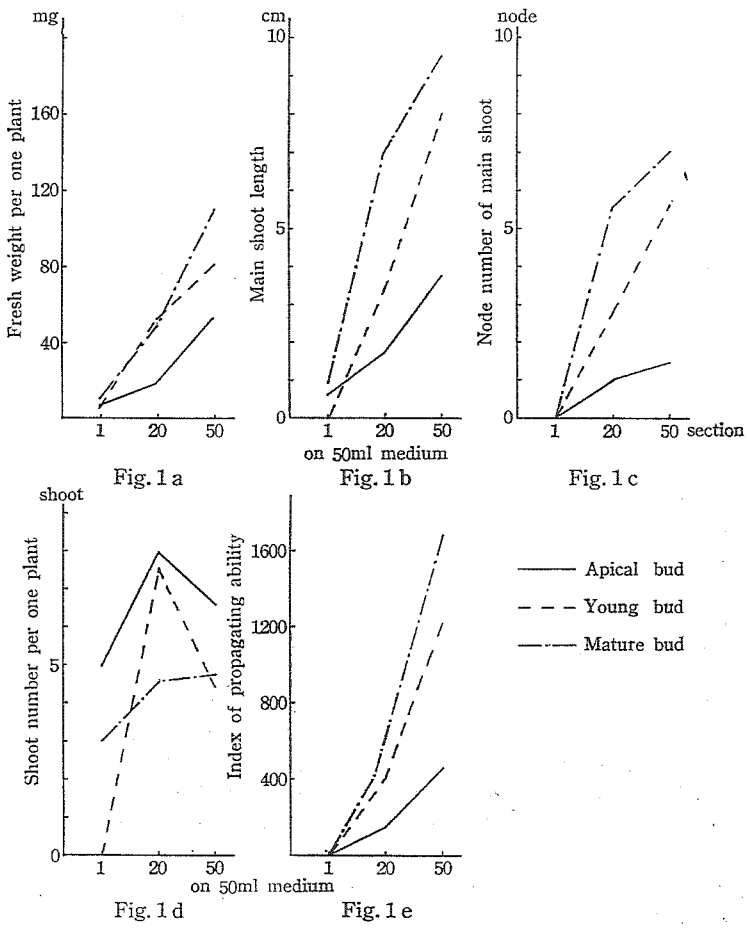

Fig. 1a Fig. 1b Fig. 1c

Fig. 1d Fig. 1e

Fig. 1. Effects of density and age of bud on shoot growth.

Procedure 2 :

Each shoot grown on the basic medium containing 0, 10^{-2} or 10^{-1} mg/l of NAA was cut into a 3 or 5 mm section with a bud, as an explant of second procedure.

These 10 explants were placed on 50 ml of the basic medium containing 0.1, 0.3, 0.6 or 1.0 mg/l of NAA and 0.1 mg/l of kinetin in 125 ml flask. The optimum concentrations of NAA and kinetin for shoot and root growth were 0.3 mg/l of NAA and 0.1 mg/l of kinetin.

Further studies of buds were made in the difference of regenerating abilities within apical, young or mature buds, on 50 ml of medium with 1, 20, and 50 bud densities.

A plant subcultured on the basic medium every three months for one year was used as the mother plant. The mother plant had a main elongated shoot 12 cm tall, 17.2 nodes and 37.4 shoots. Buds were classified into apical, young and mature buds depending on the location of buds on a shoot. The remaining shoot excised as an apical bud was separated into a distal half and a proximal half, and buds on each half were classified as the young or mature bud.

One, 20, or 50 buds were placed on two kinds of media of 50 ml in a 125 ml flask. One kind was the basic medium which induced only good shoot growth. Another was the basic medium containing 0.3 mg/l of NAA and 0.1 mg/l of kinetin which induced both shoot and root growth. Figs. 1 and 2 show the result of 40 days culture on the basic medium. Figs. 1a, 1b, 1c and 1e indicate, respectively, fresh weight per plantlet, main shoot length, node number of main shoot, and main stem node x shoot number x density as an index of propagating ability. These four figures indicate that the good shoot growth was obtained from mature buds on a medium of high bud density. The shoot number per plantlet shown in Fig. 1d shows apical and young buds in a medium of 20 bud density produced the most shoots. A plant subcultured on the basic medium for over three months seldom produced roots as shown in Fig. 3.

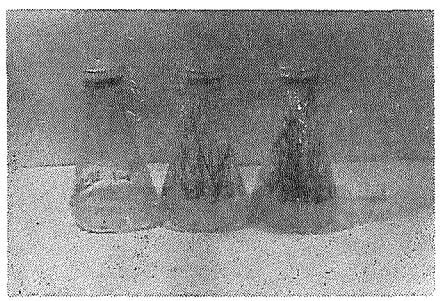

Fig. 2. Shoot growth from mature bud on the basic medium after 40 days culturing.
left : one bud in 50 ml medium
middle : 20 buds 〃
right : 50 buds 〃

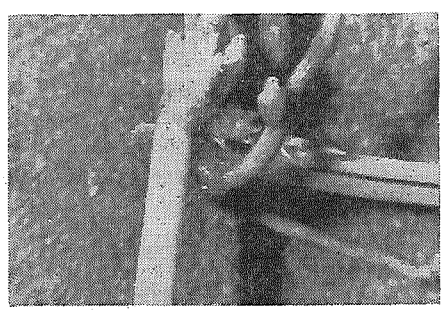

Fig. 3. Root cultured on the basic medium.

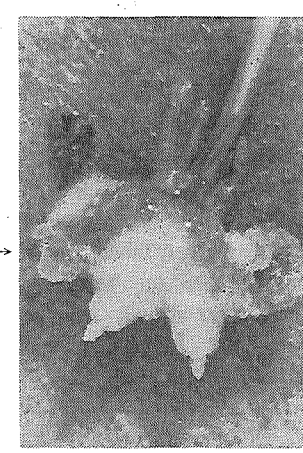

Fig. 5. Roots cultured on the basic medium containing 0.3 mg/l of NAA and 0.1 mg/l of kinetin.

Fig. 4 shows the result of bud culturing for 40 days on the basic medium containing 0.3 mg/l of NAA and 0.1 mg/l of kinetin. Figs. 4a, 4b and 4c show, respectively, fresh weight per plantlet, main shoot length and the node number of the main shoot.

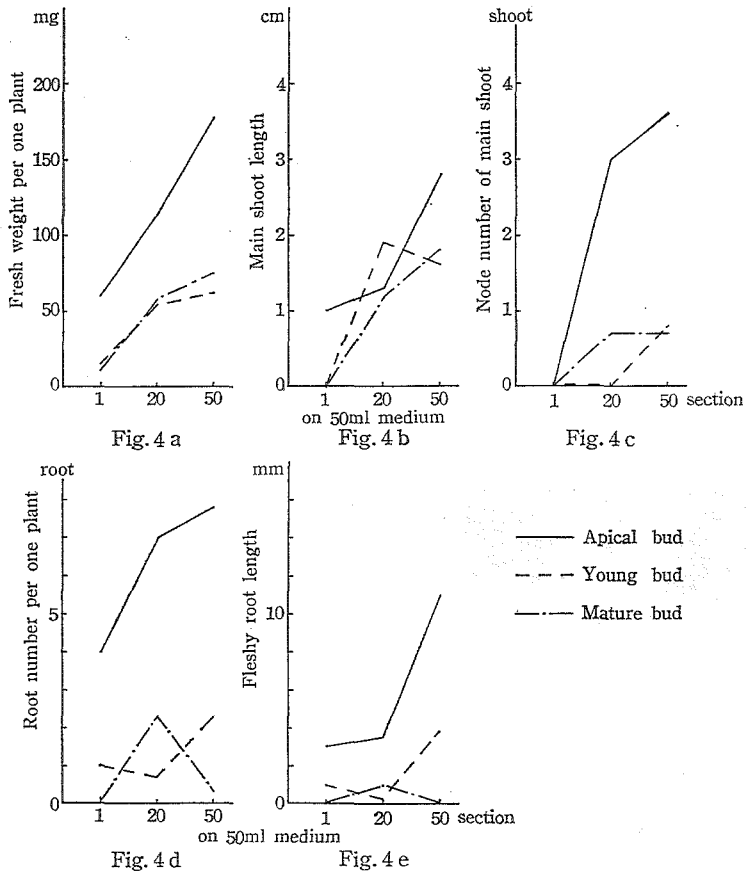

Fig. 4a Fig. 4b Fig. 4c

Fig. 4d Fig. 4e

Fig. 4. Effects of density and age of bud on shoot and root growth.

Figs. 4d and 4e show the root number per plantlet and the length of fleshy root, respectively.

These data indicate that the shoot and root growth were obtained from the apical bud cultured on the medium of high bud density.

Fig. 5 shows the root developed on this medium. This root appears very swollen and much different from the root cultured on the basic medium shown in Fig. 3. However, this root on the basic medium with NAA and kinetin will grow more slender and normal after it is transplanted to the basic medium of Procedure 3 as shown in Fig. 8.

On a medium of high bud density, a mature bud has a high potential for shoot growth (Fig. 1), and an apical bud has a high potential for shoot and root growth (Fig. 4). Promoting effects of this high density may be caused by a substance or substances differing from explants into the medium. Using NAA, we checked to determine if this substance was auxin-like or not. Both 1 and 50 buds were placed on the three kinds of medium consisting of 50 ml in a 125 ml flask. These media

were composed from the basic medium containing 0.0, 0.01 and 0.1 mg/l of NAA. Figs. 6a and 6b show the results of 20 days culturing. For this comparison only the 50 buds density on the basic medium and the single bud on the basic medium with NAA are shown. NAA did not result in good shoot growth, and the promoting effect by high bud density does not appear to be a result of NAA-like substance.

The implantation of a node on the medium was also studied. Stems with single young and mature buds were inserted in or on the medium with distal end up, proximal end up, or laid horizontally. Results are shown in Fig. 7. On the basic medium, if a stem with bud was inserted with distal end up or horizontally, shoot growth occurred, and if inserted with the proximal end up, callus was formed on the aerial proximal end and shoot growth occurred (Fig. 7).

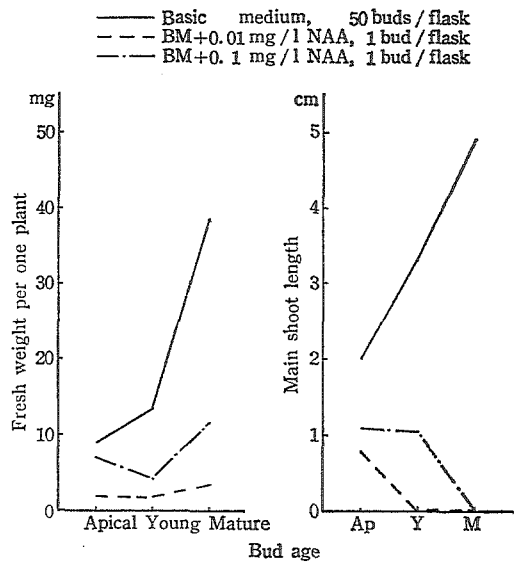

Fig. 6a Fig. 6b

Fig. 6. Effects of density and NAA on shoot growth.

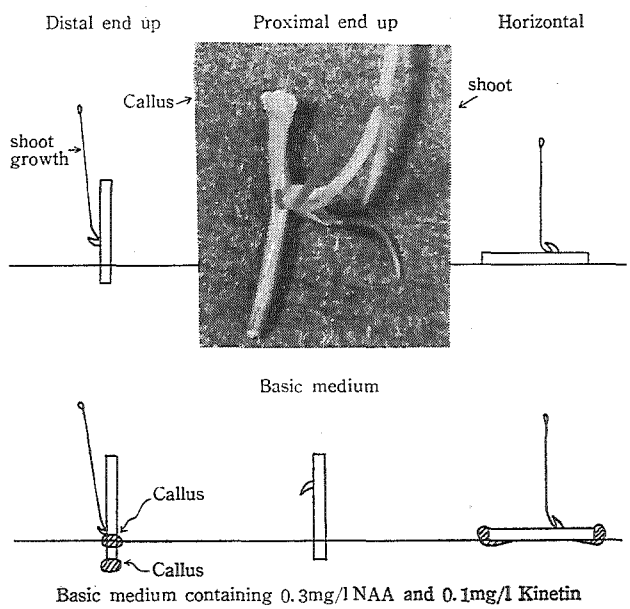

Fig. 7. Effect of inserting direction of node on shoot growth and callus formation.

If a node was inserted distal end up on the basic medium containing 0.3 mg/l of NAA and 0.1 mg/l of kinetin, callus formation occurred at the proximal end. Shoot growth

occurred from the node, and roots develop slowly from the proximal end. But when the proximal end was up there was no response. If a node was placed horizontally callus was formed on both ends, with the greater amount forming on the topside (Fig. 7).

The shoot growth of the vertical implantations of the distal and proximal ends was slowly, more slender, and with fewer nodes than that of the horizontal implanted tissue.

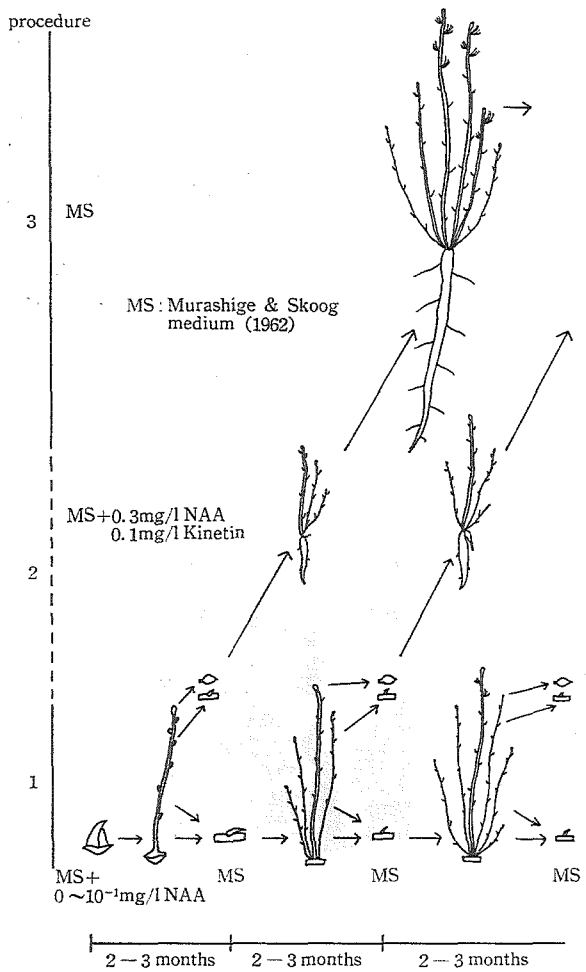

Fig. 8. Lateral bud culture.

Procedure 3 :

Five plantlets with several shoots and roots obtained from Procedure 2 were transplanted on the basic medium of 100 ml in a 500 ml flask.

Lateral bud culture, through first, second and third procedures is shown in Fig. 8.

A count of chromosomes in the root tips of several of these propagants indicated they were all diploid.

Summary

Procedures of vegetative propagation of asparagus were studied using lateral bud culture. Lateral buds excised from a spear were placed on the Murashige and Skoog's medium, the basic medium used in this study, with or without NAA. Shoots that were grown on this medium were used for further vegetative propagation. Mature buds on the proximal part of the shoot were placed on the basic medium and good shoot growth resulted. Apical and young buds on the distal part of shoots were planted on the basic medium containing NAA and kinetin. Shoot and root growth resulted. Plantlets with shoots and roots were transplanted to the basic medium in 500 ml flasks and grew to transplantable size.

A comparison of 1, 20 and 50 bud densities/50 ml of medium indicated that there was a growth-promoting diffusing-substance evident by the marked improvement of shoot and root growth at the highest bud density. It was determined that this growth promoting effect was not caused by NAA.

Horizontal placing of shoot with bud upright on the medium was more effective for shoot and root growth than either shoots implanted with distal end up or with proximal end up.

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側芽によるアスパラガスの栄養繁殖

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側芽を用いたアスパラガスの栄養繁殖の方法が 調べられた. 若茎から 切りとった 側芽を, NAA 添加, または無添加の基本培地に 植付けた. 基本培地としては, ムラシゲ・スクーグ (1962) の有機, 無機物に 30 g/l しょ糖と, 7 g/l 寒天を添加したものが用いられた. 培養後生長してきた茎を, 栄養繁殖のための材料に用いた.

茎の基部に近い部分からとった側芽を基本培地に植付けると, 茎の生長がよい. 茎の生長点や, それに近い部分からとった側芽を NAA とカイネチンをふくんだ基本培地に植付けると, 茎と根が同時に伸長する. 茎と根が伸長した小植物体は, 基本培地に移植し, 鉢上げできる大きさにまで生育させる.

50 ml の培地に 1, 20 または 50 側芽を植付け生長を比較してみると, 高密度の方ほどよく生長する. これは生長促進物質によるものではないか, と思われ, この効果は NAA ではおき代えられない.

植付けのさい, 一芽をふくんだ切片を水平位置に置床したものは, 基部側の切口を寒天内にさしたものや, 上むきにさしたものより生育がよい.