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# *In vitro* Rooting of Male and Female Asparagus Derived from Apices and Lateral Bud Explants

Sachiko MATSUBARA, Masaharu MASUDA[a] and Kazuhisa TAKAHASHI
(*Divisisn of Biological Function and Genetic Resources Science*)

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## Summary

Treatments to promote *in vitro* rooting of shoots derived from apices and lateral buds of male and female asparagus plants were studied. Effects of phytohormones, NAA and BA, IBA and Rootone (0.4% α-naphthylacetoamide) were evaluated. Apices and lateral buds placed on MS basal medium +0.3 mg·l$^{-1}$NAA and 0.1 mg·l$^{-1}$BA as a root-initiation medium for 0, 2, 3 and 4 weeks followed by transfer to MS basal medium indicated that two or three weeks treatment was the most effective on rooting of male lateral buds and female apices. Apices and lateral buds were also placed on MS medium containing 0, 0.01, 0.1, 1,0 and 10.0 mg·l$^{-1}$ of IBA. Seventy % of male lateral shoots rooted on MS medium +1 mg·l$^{-1}$IBA, but it was ineffective for female shoots. Rootone was the most effective on rooting of lateral shoots, irrespective of sexes, but ineffective on apical shoots.

## Introduction

Asparagus is a dioecious plant that it is generally propagated by seeds. Sex ratio of male and female plants is 1 : 1 and male plants have flower types that vary from staminate to hermaphroditic[11]. Since staminate plants produce high yields, there have been efforts in asparagus breeding to produce all male varieties. All male varieties are obtained by crossing females with males YY chromosomes, or by self-pollination of hermaphrodite flower having XY chromosomes[11]. The former method has not been established, because obtaining haploid plants having Y chromosome by anther culture is very difficult. The latter method is also difficult, but recently a few all male varieties were obtained by this method. On the other hand, good male plants could be vegetatively propagated by apex or lateral bud culture[1,5,6,7,8], callus culture[10,14], cell culture[12,13] and protoplast culture[2]. In practice, apex and lateral bud culture have been used, but rooting of shoots is often inefficient, even when root-promoting medium[3,4,5] was used. One of reasons for inefficiency may be the differences between sexes and ages of explants. In the present study, root-promoting treatments were tried by using shoots derived from apices and lateral buds *in vitro*.

## Materials and Methods

Axenic male and female shoots of *Asparagus offcinalis* L. cv. "Pole Tom" (Sakata Seed Co.) were used. Axenic plantlets were subcultured every two months during one year from May, 1985 to next May. In order to study the effect of rooting, combinations of NAA and BA, Indolebutylic acid (IBA) and Rootone were separately added to the basal medium of Murashige and Skoog's inorganic salts (MS)[9] plus (all in mg·l$^{-1}$) 2.0 *myo*-inositol, 0.5 nicotinic acid and pyridoxine·HCl, 0.1 thiamine·HCl, 3000 sucrose and 800 agar. Shoot and root growth was studied after 30 days of *in vitro* culture. Ten explants were used for each treatment, except 15 explants in the NAA-BA treatment.

*Effects of NAA and BA.* Apices and lateral buds were excised from axenic shoots, and

a) Division of Eco-Physiology for Crop Production.

three explants were placed in a test tube containing 10 ml of test medium. They were cultured for 2, 3, or 4 weeks and then transferred to MS basal medium.

*Effect of IBA.* Explants were cultured for one month on MS medium containing 0.01 mg·l$^{-1}$ of both NAA and BA. Shoots obtained were placed on MS medium containing 0, 0.01, 0.1 and 10.0 mg·l$^{-1}$ of IBA. Two shoots were placed in a test tube containing 10 ml medium.

*Effect of Rootone.* The basal end of shoots of one month age were dipped in Rootone powder and sticked into the basal medium. Two shoots were placed in each test tube.

## Results and Discussion

*Effects of NAA and BA.* Higher percent of apices than lateral buds grew in both male and female plants (Table 1). Fifty and 54.5% of male shoots derived lateral buds (lateral shoots) rooted with 2 and 3 week treatment on the root-initiation medium, respectively. Significant good root growth was obtained with 2 week treatment, and somewhat stunted and callus-like growth was observed with 3 week treatment. In female propagules, 50% of shoots derived apices (apical shoots) rooted with 3 week treatment, and 22−40% of apical and lateral shoots rooted, irrespective of period of treatment. Untreated explants did not root.

*Effect of IBA.* In male propagule, 70% of lateral shoots rooted on 1.0 mg·l$^{-1}$ of IBA, and 30% of both apical and lateral shoots rooted at 10.0 mg·l$^{-1}$ of IBA (Table 2). Other concentrations of IBA were ineffective. Root and shoot growth was significantly better in male lateral shoots than apical one at 1.0 mg·l$^{-1}$ of IBA. In female propagules, 30−40% of lateral shoots rooted, irrespective of concentrations of IBA, and 20% of apical shoots rooted at 0.01 mg·l$^{-1}$ of IBA. Thus IBA was ineffective on rooting of female apical propagules.

*Effect of Rootone.* Rootone was specially effective on rooting of lateral shoots. Seventy and 80% of male and female lateral shoots rooted, respectively (Table 3, Fig. 1). The number of shoots was also significantly higher in lateral shoots, although root growth was not significantly increased.

From these studies, it was revealed that lateral shoots rooted with more efficiency than apical shoots, and the difference was magnified with phytohormones. Growth of apical and

Table 1 Effect of NAA-BA treatment on rooting of apical and lateral asparagus shoots

| Explant Sex Bud | Length of treatment (weeks) | Bud yielding shoots (%) | Longest shoot length (cm) | No. shoots per propagule | Rooted shoots (%) | Longest root length (mm) | No. roots per propagule |
|---|---|---|---|---|---|---|---|
| ♂ Apical | 0 | 93.3 | 5.9$_a$* | 2.8$_b$ | 0 | — | — |
| | 2 | 100 | 4.8$_a$ | 4.4$_{ab}$ | 13.3 | 12.5$_{ab}$ | 5.5$_a$ |
| | 3 | 93.3 | 5.3$_a$ | 3.4$_b$ | 21.4 | 24.0$_{ab}$ | 2.7$_b$ |
| | 4 | 100 | 4.3$_a$ | 4.5$_{ab}$ | 26.7 | 7.8$_b$ | 1.5$_b$ |
| Lateral | 0 | 66.7 | 5.5$_a$ | 2.8$_b$ | 0 | — | — |
| | 2 | 53.3 | 5.8$_a$ | 3.6$_{ab}$ | 50.0 | 36.5$_a$ | 1.3$_b$ |
| | 3 | 73.3 | 5.0$_a$ | 4.4$_{ab}$ | 54.5 | 20.2$_{ab}$ | 1.5$_b$ |
| | 4 | 66.7 | 4.8$_a$ | 5.4$_a$ | 40.0 | 8.5$_b$ | 3.3$_b$ |
| ♀ Apical | 0 | 86.7 | 2.3$_b$ | 2.2$_b$ | 0 | — | — |
| | 2 | 73.3 | 3.5$_{ab}$ | 3.3$_{ab}$ | 36.7 | 13.3$_a$ | 1.0$_b$ |
| | 3 | 93.3 | 3.2$_{ab}$ | 6.8$_a$ | 50.0 | 13.6$_a$ | 2.0$_b$ |
| | 4 | 66.7 | 3.0$_{ab}$ | 6.7$_a$ | 40.0 | 16.0$_a$ | 5.8$_a$ |
| Lateral | 0 | 66.7 | 3.4$_{ab}$ | 4.0$_{ab}$ | 0 | — | — |
| | 2 | 73.3 | 3.7$_{ab}$ | 4.1$_{ab}$ | 36.4 | 20.8$_a$ | 2.8$_b$ |
| | 3 | 60.0 | 4.3$_a$ | 4.4$_{ab}$ | 37.5 | 8.0$_a$ | 1.0$_b$ |
| | 4 | 60.0 | 3.8$_{ab}$ | 4.9$_{ab}$ | 22.2 | 6.5$_a$ | 2.0$_b$ |

* Mean separation in columns by Duncan's multiple range test, 5% level.

lateral shoots was similar after one month culture, but lateral shoots jointed with mother stem of a few mm initiated roots from the mother stem tissue.

Rootone was found effective for lateral shoots of both male and female asparagus, whereas IBA was specifically effective for the male plant. Rootone is generally used for semi-softwood cuttings, and it contained $4\times10^{-4}$mg·l$^{-1}$ of α-naphthylacetoamide (NAa) in mineral agent. This concentration is very high for plantlets cultured *in vitro*, although it is not clear that how many NAa was absorbed into explants. Effectiveness of Rootone to explants may be continued for long duration. Therefore, shoots treated with Rootone have sometimes formed callus on the basal ends dipped in Rootone powder.

**Table 2** Effect of IBA treatment on rooting of male and female asparagus shoots

| Explant Sex Shoot* | Conc. of IBA (mg/l) | Longest shoot length (cm) | No. shoots per propagule | Rooted shoots (%) | Longest root length (mm) | No. roots per propagule |
|---|---|---|---|---|---|---|
| ♂ Apical | 0.0 | 6.2$_{ab}$** | 2.9$_{bcd}$ | 0 | — | — |
| | 0.01 | 4.0$_{c}$ | 2.2$_{d}$ | 0 | — | — |
| | 0.1 | 4.1$_{c}$ | 2.7$_{cd}$ | 0 | — | — |
| | 1.0 | 6.2$_{ab}$ | 3.8$_{abc}$ | 0 | — | — |
| | 10.0 | 5.7$_{ab}$ | 2.4$_{d}$ | 30 | 18.3$_{b}$ | 1.3$_{a}$ |
| Lateral | 0.0 | 6.4$_{ab}$ | 3.1$_{bcd}$ | 0 | — | — |
| | 0.01 | 6.4$_{ab}$ | 3.6$_{abc}$ | 0 | — | — |
| | 0.1 | 5.7$_{ab}$ | 4.0$_{ab}$ | 0 | — | — |
| | 1.0 | 7.0$_{a}$ | 4.4$_{a}$ | 70 | 37.1$_{a}$ | 1.9$_{a}$ |
| | 10.0 | 5.3$_{bc}$ | 3.0$_{bcd}$ | 30 | 18.3$_{b}$ | 1.3$_{a}$ |
| ♀ Apical | 0.0 | 3.9$_{bc}$ | 2.2$_{bc}$ | 0 | — | — |
| | 0.01 | 4.5$_{bc}$ | 2.3$_{bc}$ | 20 | 8.0$_{b}$ | 2.0$_{a}$ |
| | 0.1 | 3.3$_{c}$ | 2.3$_{bc}$ | 0 | — | — |
| | 1.0 | 6.6$_{a}$ | 3.5$_{ab}$ | 0 | — | — |
| | 10.0 | 5.5$_{ab}$ | 1.5$_{c}$ | 0 | — | — |
| Lateral | 0.0 | 5.1$_{abc}$ | 3.3$_{ab}$ | 30 | 21.7$_{ab}$ | 1.3$_{a}$ |
| | 0.01 | 5.6$_{ab}$ | 4.1$_{a}$ | 40 | 36.3$_{a}$ | 2.0$_{a}$ |
| | 0.1 | 4.7$_{bc}$ | 3.4$_{ab}$ | 30 | 36.7$_{a}$ | 1.7$_{a}$ |
| | 1.0 | 4.9$_{abc}$ | 3.6$_{ab}$ | 30 | 31.7$_{a}$ | 1.3$_{a}$ |
| | 10.0 | 5.2$_{abc}$ | 2.3$_{bc}$ | 40 | 16.8$_{ab}$ | 1.8$_{a}$ |

* Apical shoots grown from apices. Lateral shoots, from lateral buds.
** Mean separation in columns by Duncan's multiple range test, 5% level.

**Table 3** Effect of Rootone (0.4% α-naphthyl acetoamide) on rooting of male and female asparagus shoots

| Explant Sex Shoot* | Rootone treatment | Longest shoot length (cm) | No. shoots per propagule | Rooted shoots (%) | Longest root length (mm) | No. roots per propagule |
|---|---|---|---|---|---|---|
| ♂ Apical | + | 5.4$_{b}$** | 2.4$_{b}$ | 0 | — | — |
| | − | 7.4$_{a}$ | 3.1$_{b}$ | 0 | — | — |
| Lateral | + | 7.0$_{a}$ | 6.0$_{a}$ | 70 | 36.0$_{a}$ | 2.7$_{a}$ |
| | − | 6.9$_{a}$ | 3.2$_{b}$ | 20 | 22.5$_{a}$ | 1.0$_{a}$ |
| ♀ Apical | + | 5.2$_{a}$ | 2.5$_{b}$ | 0 | — | — |
| | − | 6.9$_{a}$ | 3.2$_{b}$ | 0 | — | — |
| Lateral | + | 6.0$_{a}$ | 6.1$_{a}$ | 80 | 25.3$_{a}$ | 2.1$_{a}$ |
| | − | 5.1$_{a}$ | 3.9$_{b}$ | 30 | 27.0$_{a}$ | 1.7$_{a}$ |

* See Table 2 (*).
** Mean separation in columns by Duncan's multiple range test, 5% level.

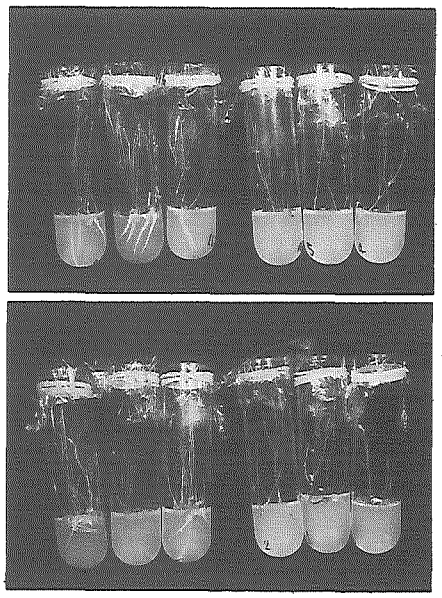

Fig. 1 Root growth of shoots treated with Rootone powder. Upper: male shoots. Lower: female shoots. Left three: lateral shoots. Right three: apical shoots.

Root-promoting effect of NAA-BA treatment was similar to that obtained in the previous treatment[5], although kinetin in the previous treatment was used.

As root-promoting treatment of male shoots, it may be recommended that lateral shoots are placed on the MS medium containing 1.0 mg·l$^{-1}$ IBA, or on MS medium after Rootone application.

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## 人工培地上でのアスパラガスの頂芽及び側芽由来の雌雄小植物体の発根

松原幸子・桝田正治[a)]・高橋和久
(生物機能・遺伝資源開発学講座)

人工培地上でのアスパラガスの頂芽及び側芽由来の雌雄小植物体の発根について，種々の植物ホルモンの効果を調べた．供試したホルモンの種類は，NAA と BA の組み合わせ，IBA，ルートン（0.4% アセトアマイドを含む）であった．

頂芽と側芽を，0.3 mg/l NAA と 0.1 mg/l BA を添加した MS 培地（発根促進培地）上で0，2，3，4週間培養し，MS 培地に移植したところ，雄側芽から伸長した苗条（側芽苗条）と雌茎頂から伸長した苗条（茎頂苗条）では2－3週間の発根促進培地培養処理で良く発根した．

MS 培地に0，0.01，0.1，1.0 および 10.0 mg/l IBA を添加した処理では，1.0 mg/l 添加処理で 70% 雄側芽苗条が発根したが，雌苗条では効果がなかった．

苗条基部にルートンを塗布し MS 培地に植え付けたところ，性に関係無く側芽苗条で高い発根率が得られたが，茎頂苗条では無効であった．

a) 作物機能調節学講座