

Studies on the Vegetative Propagation of Korean Ginseng (*Panax ginseng* C. A. Meyer)

1. Effects of the NAA Concentration and the Rooting Media on the Rooting of the Ginseng Stem Cutting

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高麗人蔘의 營養繁殖에 관한 研究

第 1 報 NAA의 濃度 및 插木用培地가 人蔘茎插穗의 發根에 미치는 影響

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ABSTRACT

To develop a new propagation method of Korean ginseng (*Panax ginseng* C.A. Meyer) by cutting, stem cuttings with leaflets obtained from ginseng seedlings were planted in rooting media treated with three levels of NAA concentration. NAA solution of 1.0 ppm was the most effective for the rooting of ginseng stem cuttings as well as for root growth after initiation of the roots from cuttings. A mixture of sand and leaf compost as a rooting medium for ginseng stem cuttings showed the best results in rooting percentage of cuttings and root growth after rooting. An acril film cap covering was very effective to prevent water loss by transpiration from the leaflets of stem cuttings and for rooting and root growth after rooting. Cuttings of leaf petiole with 5 leaflets from 2 year old ginseng plants showed good rooting as well as root growth after rooting in a mixture of sand and leaf compost treated with 1.0 ppm NAA.

INTRODUCTION

As it takes at least 3 or 6 years for one generation of a ginseng plant (*Panax ginseng* C.A. Meyer) to mature, 20 to 30 years are required to develop a new variety, and another 20 years are necessary for enough multiplication of the new variety. To solve this problem a more convenient propagation method of ginseng must be developed.

In 1963, M.K. Grushvtchkaya et al²⁾ of the CCCP reported that callus and adventitious roots

were initiated at the cut surface of a ginseng stem cutting. They²⁾ used a glass bottle cap to prevent water loss by transpiration from ginseng leaflets. But they did not use any plant growth substance for the rooting of the cuttings. In a previous study on the effects of plant growth regulator on callus induction³⁾⁴⁾ and on root initiation from ginseng stem segments, J. Jo⁶⁾⁷⁾ found that Naphthaleneacetic acid (NAA) had an excellent effect on inducing roots from ginseng stem segments. He⁷⁾ also suggested that NAA treatment should enhance the possibility of the vegetative propagation of the

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ginseng plant by stem cutting.

The present study was conducted to determine the effects of NAA and rooting media on the rooting of ginseng stem cuttings and then to define the possibility of vegetative propagation of Korean ginseng by stem cutting.

MATERIALS AND METHODS

Experiment I.

Rooting of ginseng stem cuttings in in-vitro culture: To determine the suitable concentration of NAA for rooting and to observe the rooting phase of the ginseng stem cuttings, experiment I was carried out using test tubes, 15.0x1.8cm. Both the Murashige and Skoog (MS) agar medium and the Blaydes' agar medium were used as rooting media for ginseng stem cuttings. Four rooting medias were used: An MS agar medium containing 2.0ppm NAA and three Blaydes' agar medias containing 0.5ppm NAA, 1.0ppm NAA, and 2.0ppm NAA.

The cuttings were taken from ginseng seedlings which were obtained from the ginseng embryo culture on the MS agar medium 45 days after planting. At that time, the stem length had reached about 4cm and the leaflets were fully expanded. The stem cuttings were obtained by cutting 2cm below the leaflets of the ginseng seedlings and were about 2cm long with 3 seedling leaflets. Before the cuttings were made, the seedlings were soaked in a 10% NaOCl solution for 10 minutes and washed 2 times with sterilized water to prevent contamination. The stem cuttings were lightly inserted into the agar medium and the tops of the test tubes were covered with aluminum cooking foil. 60 cuttings were taken for each treatment of NAA concentration and for the basic medium. The stem cuttings were grown in a growth chamber in which temperature was about 20°C and light intensity was about 3000 lux. Rooting percentage, number of roots and rooting stage of stem cuttings were observed 40 days after planting.

Experiment II.

Rooting of the ginseng stem cuttings in sand: The new ginseng plants obtained from the stem cuttings in nutrient agar medium had difficulty in surviving after transplanting in leaf compost, because of the soft and fragile callus formed at the base of cuttings and weak root systems. So, sand was thought to be more effective for rooting of ginseng stem cuttings and growing of ginseng plants obtained from the cuttings.

Sterilized fine sand was used as the rooting medium for the stem cuttings in experiment II. 6cm by 16cm vials were filled with sand to a height of 7.5cm. The ginseng stem cuttings were planted in sand at a depth of about 1cm. After planting, the cuttings were irrigated with NAA solution and top of vial was covered with aluminum cooking foil to prevent water loss from leaflets by transpiration. The cuttings were grown in the growth chamber under the same conditions as those of experiment I. The NAA concentrations prepared for this experiment were 0.5 and 1.0ppm, and 20 vials were used for each concentration of NAA. The stem cuttings used in experiment II were obtained from ginseng seedlings which were grown in plastic pots for 70 days. Rooting percentage and number and length of roots were measured 50 days after planting.

Experiment III.

Rooting of the ginseng stem cuttings in a mixture of sand and leaf compost: It was very difficult to control the water content of sand medium was too moist for root growth after rooting. The ginseng plants obtained from stem cuttings in sand medium had to be transplanted in a leaf compost in order to make their growth continue. This experiment was conducted to find out a way of eliminating transplanting step after rooting. A mixture of sand and leaf compost was used as a rooting medium for this purpose. This rooting medium consisted of sand and leaf compost in a ratio of 1:1. The containers used in this experiment were small plastic pots, 10cm in diameter and 10cm

in height. The stem cuttings used were taken from ginseng seedlings grown in plastic pots for 80 days, at which time the leaflets were fully expanded and the stem length was about 5cm. The stem cuttings were 3cm in length with 3 seedling leaflets. After filling plastic pots with rooting medium, the stem cuttings were planted in the rooting medium and irrigated with NAA solution. After irrigation, the exposed portion of cuttings was covered with an acril film cap to prevent water loss by transpiration from the leaflets (Fig. 1). NAA concentrations in this experiment were 0.5, 1.0 and 2.0ppm. For each NAA concentration, 30 cuttings were tested. The rooting percentage, the number of roots and the rooting stage were observed 35 days after planting.

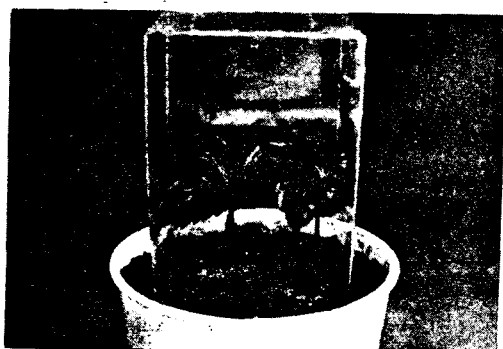


Fig. 1. The stem cuttings obtained from ginseng seedlings were planted in a mixture of sand leaf compost. The cuttings were then irrigated with an NAA solution and covered with an acril film cap to reduce transpiration from the cutting leaflets.

RESULTS AND DISCUSSION

1. Rooting of ginseng stem cuttings in in-vitro cultures:

The effects of the NAA concentrations on the rooting of ginseng stem segments in in-vitro cultures were studied⁷⁾ and it was found that a 0.5ppm NAA or higher concentration had an effect on rooting from the ginseng stem segments, but a callus was induced from the roots at the concentra-

tion of 2.0ppm. There levels of NAA concentration, 0.5, 1.0 and 2.0ppm, were studied in this experiment.

The effects of the NAA concentrations and the kind of media on the rooting percentage and the number of roots from the stem cuttings 40 days after treatment are shown in table 3. The percentages of rooting was higher at 0.5 and 1.0ppm NAA than that at 2.0ppm NAA. But there was no significant difference in rooting percent between the two basic mediums. In each treatment, the rooting percentage at 40 days after planting the cuttings on the media was about 70 to 30%. Some cuttings showed root initiation 15 days after the planting of the cuttings in the media. The average

Table 1. Effect of NAA on the rooting percent, the days for rooting and the number of roots of the ginseng stem-cuttings in-vitro.

| Kind of Mediums | NAA ppm | Rooting Percent | Days for Rooting | Number of Roots |
|-----------------|---------|-----------------|------------------|-----------------|
| BL | 0.5 | 87.2 a | 31.4±11.0 | 4.3 a |
| BL | 1.0 | 87.8 a | 29.9± 8.3 | 5.0 aa |
| BL | 2.0 | 72.7 b | 32.1± 6.5 | 4.8 a |
| MS | 2.0 | 72.2 b | 35.5± 6.7 | 29. b |

BL : Blaydes' medium

MS : Murashige and Skoog medium

Different letters indicate the significant difference at the 5% probality level by Duncan's Multiple Range Test.

number of days required for the rooting of the cuttings was the least in the Blaydes' medium containing 1.0ppm NAA and was the highest in the MS medium containing 2.0ppm NAA, which caused larger callus formation. Two kinds of rooting stages were observed in this experiment. Some cuttings formed thin-layered callus on their cut surface and then roots were initiated, but others produced larger callus, more than 5mm in diameter, and roots were induced after callus development. The latter generally required a longer period of time for the rooting of the cuttings than the former. The rooting stage of the cuttings planted on Blaydes' medium containing 0.5 and 1.0ppm NAA followed

the former pattern and those on the medium containing 2.0ppm NAA followed the latter. The number of roots was slightly higher for the cuttings planted on the Blaydes' medium containing 1.0ppm NAA compared with those on the Blaydes' medium containing 0.5 or 2.0ppm NAA, but there were no significant differences among them. On the other hand, the number of roots was significantly lower for the cuttings planted on the MS medium containing 2.0ppm NAA than those on other mediums and NAA concentrations. Considering the results of this experiment, 1.0ppm is appeared to be the optimum concentration of NAA for the rooting of ginseng stem cuttings.

2. Rooting of ginseng stem cuttings in sand:

The effect of the NAA concentrations on the rooting percentage of cuttings in sand medium is shown on table 2. The rooting percentage of the ginseng stem cuttings irrigated with 1.0ppm NAA solution was significantly higher than that with 0.5ppm NAA, but the root growth, number of roots and length of roots were slightly better at 0.5ppm than 1.0ppm NAA. Some cuttings showed root initiation without callus formation, but almost all of cuttings formed relatively thin and white callus which was induced on the cut surface of stem cuttings in the in-vitro culture. The callus of cuttings in the sand medium was very hard and tight.

Table 2. Effect of NAA on the rooting percent, the number of roots and the length of roots of the ginseng stem-cutting when the sand was used as the rooting media of cuttings.

| Concentration of NAA (ppM) | Rooting Percent (%) | Number of Roots | Length of Roots(mm) |
|----------------------------|---------------------|-----------------|---------------------|
| 0.5 | 76.7 b | 5.70 a | 6.27 a |
| 1.0 | 97.1 a | 5.18 a | 5.35 a |

In this cutting experiment, glass vials were used as containers and the top of vials were covered with aluminum cooking foil to prevent the cuttings from wilting. The moisture content of the sand

medium was in the state of saturation and did not change even after root initiation. Root growth after initiation was slower and poorer in sand medium than that in a mixture of sand and leaf compost (Experiment II). The most suitable moisture content for the best root growth of a ginseng plant, was 60% of the water holding capacity of the soil (data not shown). Root growth was significantly inhibited by higher moisture content in soil. It was very difficult to adjust the moisture content of a sand media in glass vials for root growth after initiation, because saturation is needed for root initiation but lower moisture is needed after root initiation. As in-vitro culture, the ginseng plants obtained from cuttings in the sand media had to be transplanted to leaf compost for continued growth, but the growth of seedlings from cutting was very poor after transplanting.

3. Rooting of ginseng stem cuttings in the mixture of sand and leaf compost:

The rooting percentage of cuttings planted in the mixture of sand and leaf compost without NAA treatment was 50%, but the cuttings treated with a 0.5ppm NAA solution or with higher concentrations (1.0 and 2.0 ppm) showed 100% rooting (Table 3). The leaflets attached to the cuttings without NAA treatment, turned red at the margins about 20 days after the cuttings had been planted in the rooting medium. The leaflets which turned red failed to root and wilted. The number of roots was significantly increased when cuttings were treated with a 2.0ppm NAA compared with those treated with lower concentrations of NAA or without NAA. There was no significant difference in number of roots between the cuttings treated with 0.5 and 1.0ppm NAA and the control plot. The length of the roots was significantly increased when the cuttings were treated with 1.0ppm NAA. NAA concentrations higher or lower than 1.0ppm were less effective for elongation of roots, and root length was significantly decreased for the cuttings without NAA treatment (Table 3 and Fig. 3). The cuttings planted in the mixture of



Fig. 2. Good root formation was shown in the cuttings planted in the mixture of sand and leaf compost but all of the roots from the ginseng stem cuttings were adventitious roots and it is unknown whether a rhizome have formed or not.

Table 3. Effect of NAA on the rooting percent, the number of roots and the length of root of the ginseng stem-cutting when the mixture of sand and leaf compost was used as the yooting media of cuttings.

| Concentrations of NAA (ppm) | Rooting Percent(%) | Number of Roots | Length of Roots(mm) |
|-----------------------------|--------------------|-----------------|---------------------|
| 0.0 | 50.0 | 7.58 b | 17.4 c |
| 0.5 | 100.0 | 6.57 b | 20.6 b |
| 1.0 | 100.0 | 7.44 b | 24.1 a |
| 2.0 | 100.0 | 9.50 a | 19.7 b |

sand and leaf compost also showed two kinds of rooting phase. Some cuttings formed small (diameter less than 3mm) and round calluses on their cut surface and others formed large (diameter more than 5mm) and cone-type calluses on their bases.

The root growth after the rooting of the ginseng

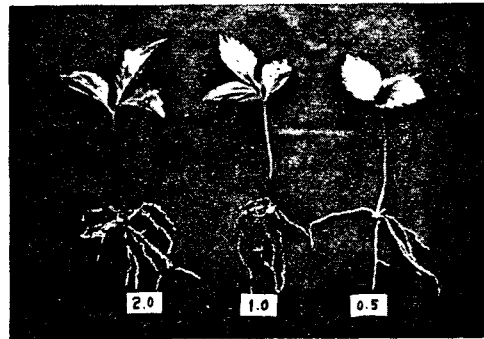


Fig. 3. The effect of different NAA concentrations on the root growth of the ginseng stem cuttings planted in a mixture of sand and leaf compost.

stem cuttings was significantly increased when a mixture of sand and leaf compost was used as rooting media of the cuttings. The moisture content of the mixture of sand and leaf compost could be easily adjusted for the rooting of the cuttings as well as root growth after initiation of the roots from cuttings. Because the moisture content inside a acril film cap could be easily adjusted by making small holes covering the above ground portion of the stem cuttings with acril film cap was more effective way to prevent water loss by transpiration than sealing the top of the glass vials or using a glass bottle to cover cuttings as described by Grushvtchkaya.²⁾

The cuttings of leaf petioles with 5 leaflets of 2 year old ginseng were planted in a mixture of sand and leaf compost media and irrigated with 1.0ppm NAA solution. The petiole cuttings showed 100% rooting, the average number of roots from petiole cutting was 7.8 and the average length of roots was 9.0mm 50 days after planting. The root growth of petiole cuttings was significantly slower than that of stem cuttings of seedlings.

Grushvtchkaya²⁾ had reported that the roots formed from stem cuttings were adventitious roots and rhizome was not induced. In this experiment, all of the roots induced from the cuttings were also adventitious roots, although it is unknown whether rhizomes have formed and new shoot

primodias developed. Although shoot primodias and shoots were induced from callus or embryos by the treatment of auxin and cytokinin together, it is of question whether rhizome and new shoot primodias could be formed in ginseng plants obtained from cuttings or not.



Fig. 4. Cuttings of the leaf petiole with 5 leaflets from 2 year old ginseng plants also showed good rooting 50 days after planting in a mixture of sand and leaf compost treated with a 1.0ppm NAA solution.

摘 要

高麗人蔘의 挿木에 의한 營養繁殖方法을 開發하고자 完全히 展葉된 3枚의 小葉을 附着한 苗蔘莖을 挿穗로 取하여 NAA의 處理濃度가 이들 挿穗의 發根에 미치는 影響을 調査하는 한편 挿木用土 및 挿木方法이 發根 및 發根後의 根生長에 미치는 影響을 調査하였다.

NAA는 挿木後 灌水時 NAA 溶液으로 灌水하였던 바 1.0 ppm 溶液의 灌水가 發根率 및 發根後의 根生長에 가장 效果의 있었으며 또한 挿木用土로는 腐葉土와 모래의 混合土가 가장 理想의 이었다.

挿木後 挿穗에 附着된 葉에서의 蒸散을 抑制하고

挿穗의 枯死를 防止하기 위해서는 Acril film으로 만든 덮개로 挿穗의 地上部를 싸워 주는 것이 挿穗의 發根에는 勿論 특히 發根後의 根生育에 效果의 이었다.

한편 5枚의 小葉이 附着되어 있는 2年生 人蔘의 葉柄을 挿穗로 하여 腐葉土와 모래의 混合土에 挿木하고 1.0ppm의 NAA 溶液을 灌水하였던 바 역시 良好한 發根率과 根生長을 보였는데 根의 生長量은 苗蔘에서 取한 莖挿穗보다는 약간 不進하였다.

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