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Induction of Callus and Organ in Tissue Culture of Ginseng (*Panax ginseng* C. A. Meyer)

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高麗人蔘의 組織培養에 의한 캘러스 및 器官分化

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Abstract

Calli and leaflets of ginseng (*Panax ginseng* C.A. Meyer) were cultured on ½MS media supplemented with kinetin, 2 iP, NAA, 2,4-D and IBA to assess their capacity to regenerate embryoids and organs.

Root calli produced numerous embryoids and shoots in ½MS medium supplemented with 2 mg/ℓ NAA and 2mg/ℓ 2iP, and the combination of 2 iP and NAA was more effective than the combination of kinetin and NAA in induction of embryoid and shoot from root calli. Culture of leaflet in the medium supplemented with IBA resulted in profuse root regeneration.

Introduction

Ginseng(*Panax ginseng* C.A. Meyer) is a perennial herbaceous plant which grows very slowly. Flowers begin to bloom in most 3-year-old plants but it is general practice to collect the seeds once from 4-year-old plants. The embryo in a seed is not mature yet even though the berry is ripe. Under natural conditions, it takes 21 months to germinate from this immature embryo. It is possible to use the tissue culture techniques for large scale propagation and breeding of ginseng. Therefore,the formation of organs in cultured tissues and leaflet cuttings of ginseng signifies simply a potential of clonal propagation of ginseng. It has been also demonstrated in a wide range of plant species that the development of organs in cultured tissue is generally dependent on the relative level of growth regulators in the medium.

The development of organs from calli and stem cuttings of ginseng has been reported by many authors[1-8]. However, the requirement for various kinds of plant growth regulators in organ formation was not examined. The objectives of this study were to establish ginseng tissue culture and to select conditions favourable for inducing root and shoot from calli and leaflet cuttings of ginseng.

Materials and Methods

Roots of 6-year-old ginseng(*Panax ginseng* C.A. Meyer) were surface-sterilized for 20 min., with shaking, in 7% calcium hypochlorite solution and rinsed 4 times with sterile distilled water. For callus induction, the segments of root with cambial cells were cultured on the basal medium with 0.1 to 8 mg/ℓ of 2,4-D in the dark for 3 months at 25 °C. The basal medium used was a modified formulation from Murashige and Skoog, and hereafter designated as MS medium. The modifications were: 100 mg/ℓ myo-inositol, 0.5 mg/ℓ nicotinic

acid. 0.5 mg/ℓ pyridoxine HCl, 0.1 mg/ℓ thiamine HCl, 2 mg/ℓ glycine, 5 g/ℓ yeast extract, 30 g/ℓ sucrose, 2 g/ℓ casein hydrolysate, and 10 g/ℓ agar. The pH was adjusted to 5.8 before autoclaving.

For organ formation from callus, a basal medium containing one half of Murashige and Skoog salts(½MS) was used and plant growth regulators, kinetin, 2 iP, BA, NAA and GA were added in various combinations. For induction of roots from the cuttings, leaflecs of 1- and 3-year-old ginseng were cutted and then cultured on the sterilized vermiculite media supplemented with the solutions of NAA, IBA, 2,4-D and GA for 3 months. The organ formation cultures were placed in a 12 hours light/12 hours dark cycle at 25 °C.The calli and cuttings were scored for frequency of root and shoot formation at intervals of one month.

Results and Discussion

(a) Induction of callus

The segments of root were inoculated on the Murashige and Skoog's basic medium supplemented with different concentrations (0.1-8 mg/ℓ) of 2,4-dichlorophenoxyacetic acid(2,4-D) for induction of callus. Formation of somatic callus started comparatively early. About 15 days after culture, calli were visible arising at the uninjured cambial cells of the cut surface of root. Regardless of the parts they arose, calli were formed more profusely on the media with 5 and 8 mg/ℓ 2,4-D. The tissues were soft and friable and comprised a wide variety of cell shapes and sizes.

(b) Root and shoot formation in segments and calli

The effect of cytokinin and auxin on root and shoot formation from segments and calli of ginseng root was tested in the presence of different concentrations of kinetin and NAA. None of two growth regulators, kinetin and NAA, brought about embryoid formation, however, many segments and calli formed roots only. Root development was localized on the medium with more than 1 mg/ℓ NAA and after 8 weeks the roots covered the entire surface of the segments and calli. A most striking effect of NAA on the initiation and development of roots was observed in root segments, and this effect is primarily dependent upon the concentration of NAA although it is influenced by other factors, such as cytokinin level in the nutrient medium. The combination of 1 mg/ℓ kinetin and 5 mg/ℓ NAA was more effective than was the other in promoting root formation. More than 2 mg/ℓ kinetin tended to inhibit root formation.

From the present studies, we found that kinetin was not effective for embryoid formation from the segments and calli of ginseng root. Therefore, further experiments were carried out with media containing other cytokinins, 2 iP and BA, and GA. Embryogenic calli were induced from the segments of root and cotyledon.

Root calli were cultured on ½MS media supplemented with combinations of 2 iP + NAA for embryoid

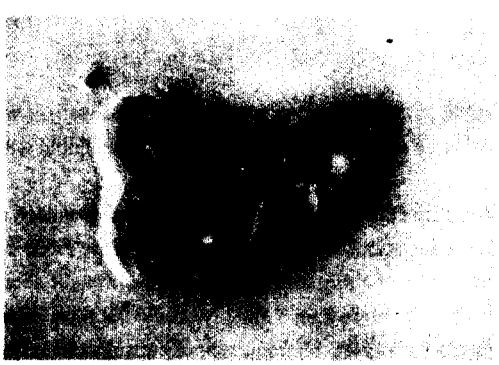

Fig. 1. Various stages of embryoids induced from ginseng root callus.

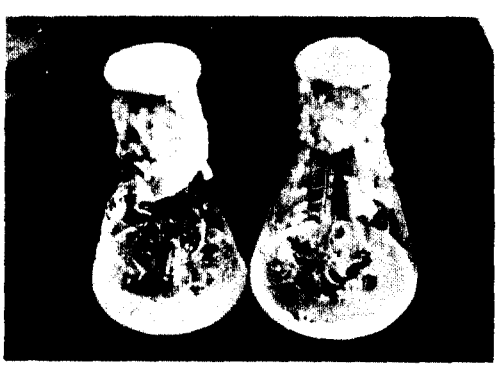

Fig. 2. Plantlets induced from ginseng root calli.

formation. Embryoids were formed 5 months after culture in root calli. Besides normal-looking embryoids various malformed embryoids were observed(Fig. 1). Initially appearing as white small globular masses, the embryoids passed through successive developmental stages and ultimately gave rise to plantlets(Fig. 2). The pattern of their formation from root calli and their changing shapes were quite similar to those reported by Chang and Hsing[9,10]. In many cases, more than one shoot developed from one embryoid. These may have been derived from numerous adventitious shoot-buds in one embryoid.

To determine the optimum levels of 2 iP for embryoid and shoot formation, different concentrations of 2 iP were incorporated into ½MS medium with 2 mg/ℓ NAA, and the root calli were cultured on these media for 6 months(Table 1). The calli cultured on the media without 2 iP and NAA did not exhibit embryoid and shoot formation. When root calli were cultured on the media containing 2 iP and NAA, the frequency of calli exhibiting embryoid and shoot formation was increased with increasing 2 iP concentrations. High percentage of embryoid and shoot formation occurred in the medium supplemented with the combination of 2 mg/ℓ 2 iP and 2 mg/ℓ NAA. But only shoot formation was less frequent. On the same medium, development of the embryoid was generally arrested at the globular stage.Further development of these embryoids occurred on the medium supplemented with the combination of 1 mg/ℓ BA and 1 mg/ℓ GA, which was developed by Chang and Hsing[9,10] for the growth of embryoids induced from 8-month old callus tissue of ginseng root. Some of the embryoids swelled considerably, callused and ultimately became transform into a mass of callus. These may have been caused by the addition of NAA to the media. Jhang *et al.*[2], on the other hand, reported that plantlets would occasionally form when Korean ginseng suspension cultures were grown for approximately 16 weeks in Prairie Regional Laboratory B5 medium with 12 ppm of NAA.

In these experiments, we have found that the combination of 2 iP and NAA is more effective than the combination of kinetin and NAA in induction of embryoid and shoot from root calli.

Table 1. Frequency of embryoid and shoot formation in root callus of *Panax ginseng* cultured in different concentrations of 2 iP and NAA

Concentration (mg/ℓ)		No. of calli	No. of calli with		Total	Per cent
2iP	NAA	cultured	Embryoid	Shoot		
0	0	60	0	0	0	0
0.1	2.0	63	5	0	5	8.0
1.0	2.0	60	3	0	3	5.0
2.0	2.0	102	21	6	27	26.5
5.0	2.0	84	9	12	21	25.0

(c) Root formation in the cuttings of ginseng leaflets

In order to clarify the requirement for exogenous auxin in root formation from ginseng leaflets, 1-year-old ginseng leaflets with petiole were cutted and then cultured on the vermiculite media supplemented with different concentrations of NAA, IBA and 2,4-D.

About 2 weeks after culture, roots were visible arising at the distal ends of leaf cuttings, and produced in variable rates when auxins were applied in different concentrations to the vermiculite medium(Table 2). The vermiculite media with IBA, 2,4-D and low concentrations of NAA, and without auxins brought about root formation, however, the cuttings cultured on the media with more than 5 mg/ℓ NAA did not form roots. When leaf cuttings were cultured on the vermiculite media with IBA, the frequency of cuttings exhibiting root formation was increased with increasing IBA concentrations. In the presence of 1 and 2 mg/ℓ 2,4-D the frequency of root development was relatively low. When the same concentration of NAA was substituted

Table 2. Frequency of root formation in the cuttings of 1-year-old ginseng leaves cultured in different concentrations of NAA, IBA and 2,4-D

Growth regulator	Concentration (mg/ℓ)	No. of leaves cultured	No. of leaves with root formation	Per cent
Non-treated	0	60	30	50
NAA	1.0	30	18	60
	2.0	30	15	50
	5.0	30	0	0
	10.0	30	0	0
IBA	1.0	30	12	40
	2.0	30	24	80
	5.0	30	24	80
	10.0	30	30	100
2,4-D	0.1	30	21	70
	0.5	30	24	80
	1.0	30	6	20
	2.0	30	3	10

Table 3. Frequency of root formation in the cuttings of 3-year-old ginseng leaflets cultured in different concentrations of IBA and GA

Concentration (mg/ℓ)		No. of leaflets cultured	No. of leaflets with root formation	Per cent
IBA	GA			
1	2	58	14	24.1
2	2	64	20	31.3
5	2	66	32	48.5
10	2	49	14	28.6

* All of the leaflets non-treated were dead.

for IBA, root formation was completely suppressed. Although root formation was induced by auxin alone, the frequency and morphology of roots formed were found to depend upon the type and concentration of auxins used. In the presence of 2,4-D, the cuttings of 1-year-old ginseng leaflets usually developed short and thick roots, and these roots formed became transform into a mass of callus. Many roots were formed in the presence of 2, 5 and 10 mg/ℓ IBA or 1 mg/ℓ NAA, but the growth of root formed was more vigorous in the presence of IBA than that in the presence of NAA and 2-4-D.

Ginseng leaves are palmately compound with a little longer petiole whorled on the tip of the stem. The number of leaves varies by the age of the plants. Yearlings have one leave with 3 leaflets and the older than 3-year-old plants have 4 and 6 leaves with 5 leaflets. For the propagation of ginseng, it is more effective to culture the cuttings of leaflets of the older than 3-year-old plants. The requirement for IBA in root formation from the cuttings of 3-year-old ginseng leaflets was tested in the presence of different concentrations of IBA and 2 mg/ℓ GA for 3 months(Table 3). The effects of IBA on the root formation from the cuttings of 3-year-old ginseng leaflets were similar to those on the cuttings of 1-year-old ginseng leaflets(Fig. 3). But the cuttings of 3-year-old ginseng leaflets gave a lower frequencies of root formation than those of 1-year-old

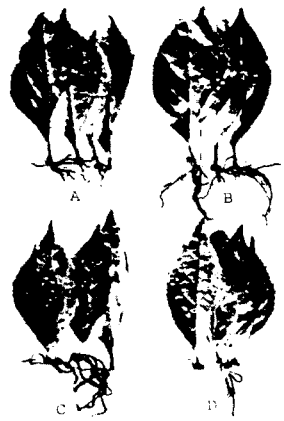

Fig. 3. Roots were formed from the cuttings of 3-year-old ginseng leaflets cultured in different concentrations of IBA.
A: 1 mg/ℓ IBA, B: 2 mg/ℓ IBA,
C: 5 mg/ℓ IBA, D: 10 mg/ℓ IBA

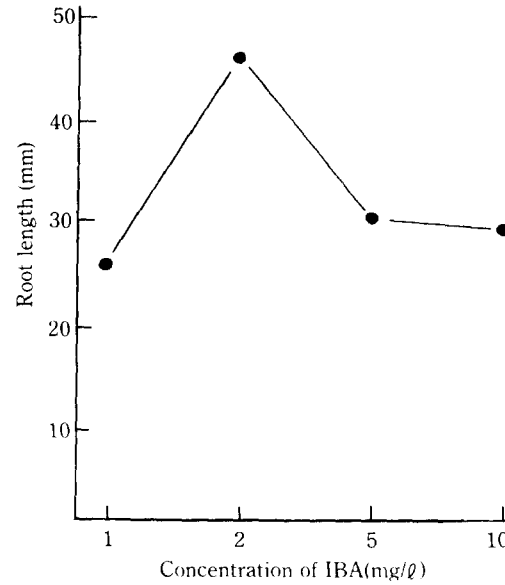

Fig. 4. Length of root derived from 3-year-old ginseng leaflet cultured in different concentrations of IBA.

ginseng leaves. The root growth, number of root, and length of root were better in the presence of 2 mg/ℓ IBA than the other concentrations of IBA(Fig. 4). In this experiment, all of the roots formed from cuttings of ginseng leaflets were adventitious roots and rhizome was not induced.

Grushvtchkaya *et al.*[7], not using any growth regulators in the cuttings of ginseng stem, produced many adventitious roots. Jo[8], on the other hand, reported induction of roots from ginseng stem cuttings using the rooting media supplemented with different concentrations of NAA. It is of question whether new shoot primordia and shoot can be formed in ginseng plants obtained from leaflet cuttings or not.

摘 要

人蔘의 優秀系統 早期增殖 硏究의 一環으로 人蔘의 뿌리 由來 Callus 및 小葉을 Kinetin, 2ip, NAA, 2,4-D, IBA 等의 植物生長調節物質을 여러가지 濃度로 달리 添加한 培養基에서 培養하였던바 그 結果를 要約하면 다음과 같다.

1. 人蔘의 뿌리由來 Callus의 胚狀體 및 地上部 分化率은 NAA 2mg/ℓ, 2iP 2mg/ℓ 添加培養基에서 가장 높았으며, 2iP가 Kinetin보다 더 效果的이었다.
2. 人蔘의 小葉器官의 뿌리分化率은 IBA 2～10mg/ℓ 添加培養基가 NAA 및 2,4-D 添加培養基보다 더 높았다.
3. 小葉의 뿌리分化率은 年生間에 差異가 있었으며, 3年生 小葉이 1年生 小葉보다 낮은 傾向을 보였다.

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