MASS PRODUCTION OF A RARE AND ENDANGERED SPECIES, *ASTRAGALUS MEMBRANACEUS* VAR. *ALPINUS* NAKAI, THROUGH AXILLARY BUD CULTURE AND IN VIVO ROOTING TEST

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Introduction

Astragalus membranaceus var.*alpinus* Nakai is a native plant to Jeju Islands (South Korea). This rare species is growing at 1,600 m above sea level in Halla mountain. In Jeju Islands, it is assumed that the number of native communities is 5~10. This plant has high scientific value and it was designated as a critically endangered by IUCN. This study was conducted to search the suitable propagation conditions by axillary bud culture and to identify the rooting characteristics of *A. membranaceu*s via rooting test.

Materials and Methods

Plant materials

Explants of *A. membranaceus* were provided from Mt. Halla located at Jeju Islands in Korea. The plantlets were firstly cultured on MS medium. After first culturing, 7-week axillary buds were sub cultured and tip stems were maintained to produce shoots and roots. The length of explants with all leaves was 2cm in *in-vitro* culture test and 3.5cm in *in-vivo* rooting test, respectively. All tests were done in laboratory conditioned at 25±1 °C 16hr fluorescent light (40μmol/m^2/s) and 8hr dark.

Axillary bud culture

Nodal segments for selecting suitable medium were incubated in two kind of medium, MS and WPM, to which various kinds of cytokinin (Kinetin, BAP, Zeatin; each 0.2, 0.5, 1.0 mg.L^{-1}) had been added. Fifty explants were used per treatment. After 4 weeks, the length of explants, number of new shoot, total leaves and callus induction were measured.

Rooting test

In-vitro rooting test

Media screening for *in-vitro* rooting tests was firstly done and WPM medium was selected. On this medium, auxin hormones such as NAA, IAA, IBA, 2.4-D were added to investigate the optimal culture condition. The concentration of these hormones was 0.1, 0.3, 0.5, 1.0, 3.0 mg.L^{-1} and total of 20 explants were plated. After 6 weeks, the number of roots, root length and root status were measured.

In-vivo rooting test

In *in-vivo* tests, hormones of NAA, IAA, IBA were used with concentrations of 50, 100, 300, 500mg$.L^{-1}$. Shoots of 3.5cm were planted in plastic pots (length 45cm * width 25cm * height 10cm) covered with transparent acrylic board and filled with instant bed soil. After 6 weeks, the number of roots, root length and root ratio were investigated from 20 explants. The humidity was 90~95% in first 3 days and after that irrigation was done once in every 3~4 days.

Acclimatization

Rooted plants were replanted in pots filled with instant bed soil and transferred under shading nursery. 4 weeks after, growth status of all tested plants was observed

Results and Discussion

Axillary bud culture

After 4 weeks of culture, Effective basic medium was MS medium for propagation of *A. membranaceus*. In MS medium, the length of explants was longer 2 times than in WPM medium. Nodal segments cultured on MS medium with different cytokinins and the results were shown in the (Table.1).

Table 1. Effect of plant hormones on *in-vivo* propagation in *A. membranaceus*.

Plant hormones ($mg.L^{-1}$)		Length of explant(cm)	No. of shoot(EA)	No. of leaves (EA)	Callus induction
Cont.		2.1	-	10.7	x
Zeatin	0.2	4.1	1.2	10.5	O
	0.5	6.7	0.8	23.9	
	1.0	5.6	0.4	22.7	
BAP	0.2	5.0	0.4	32.6	O
	0.5	4.5	2.0	23.9	
	1.0	5.1	1.8	22.9	
Kinetin	0.2	5.8	0.2	18.1	O
	0.5	5.9	0.8	17.0	
	1.0	5.4	0.6	22.1	

Rooting test

In-vitro rooting test

The rooting rate was doubled high in NAA 0.1 mg.L^{-1} as 40% comparing to hormone non treated (Table 2). The numbers of roots were relatively good in NAA treatments and the root length was best in the treatments of IBA 1.0~3.0 mg.L^{-1} (Fig 1).

Table 2. *In-vitro* rooting rate of *A. membranaceus* (WPM Medium).

Cont.	NAA (mg.L^{-1})					IAA (mg.L^{-1})					IBA (mg.L^{-1})					2,4-D (mg.L^{-1})
	0.1	0.3	0.5	1.0	3.0	0.1	0.3	0.5	1.0	3.0	0.1	0.3	0.5	1.0	3.0	0.1 ~ 3.0
20%	40 %	20 %	25 %	20 %	25 %	10 %	10 %	5%	-	-	10 %	10 %	20 %	20 %	-	-

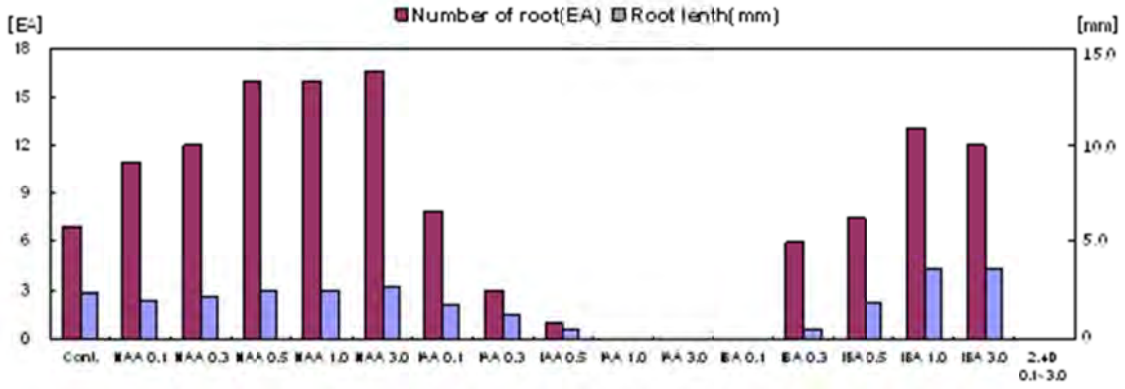

Figure 1. Rooting characteristics of *A. membranaceus* (*in vitro*).

In-vivo rooting test

The rooting rate was doubled high in IBA 500 mg. L^{-1} as 40% comparing to hormone non-treated (Table 3). The numbers of roots were relatively good in NAA treatments and the root length was best in the treatments of IBA (Fig 2, Fig 3(Left)).

Table 3. *In-vivo* rooting rate of *A. membranaceus*.

Cont.	NAA (mg.L^{-1})				IAA (mg.L^{-1})				IBA (mg.L^{-1})			
	50	100	300	500	50	100	300	500	50	100	300	500
30%	60%	45%	50%	30%	25%	45%	35%	45%	60%	55%	55%	70%

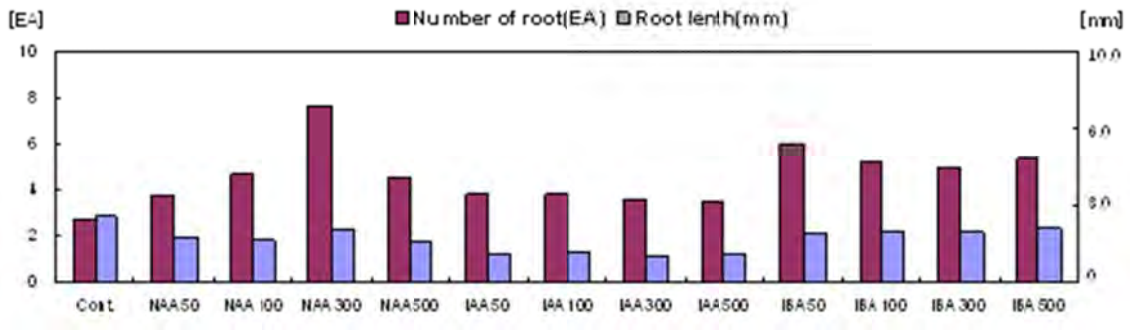

Figure 2. Rooting characteristics of *A. membranaceus* (*in-vivo*).

Acclimatization

In-vivo rooted plants were more vigorous than in-vitro ones. After 6 days of acclimatization, they seemed to shown as naturally grown with good growth form and 100% survival rate. In the next spring, new shoots were emerged from all survived plants. The average height was 20cm and it grew to 45 cm 6 month later (Fig 3(right), Fig 4).

Figure 3. Left: *In-vitro* rooted *A. membranaceus*, Right: *In-vivo* rooted *A. membranaceus*.

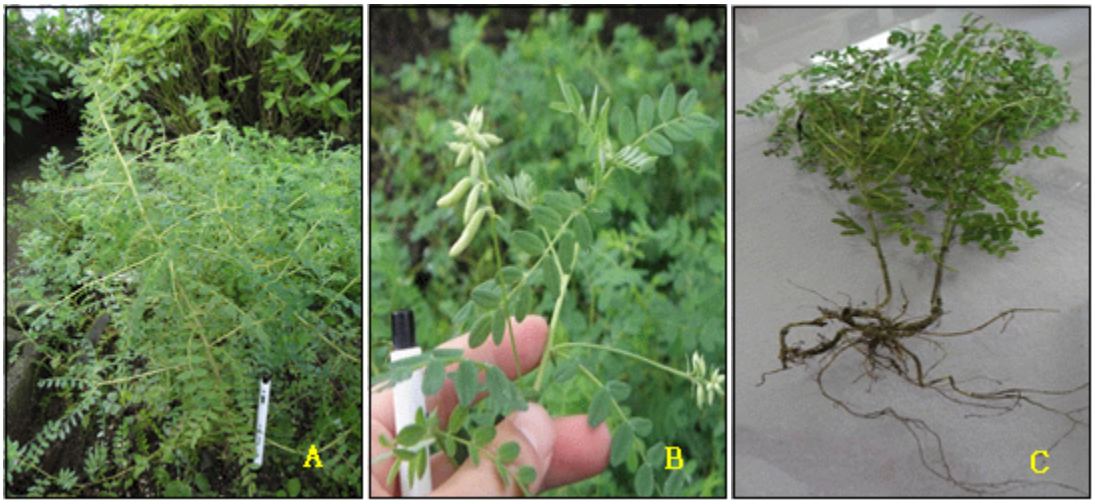

Figure 4. Vigorous grown of *A. membranaceus* in via *in vivo* rooting (A: flower bud, B: root) and naturally grown of *A. membranaceus* on nursery (C).

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