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# Comparison of Inoculation Effects for Different Seed and Nodule Sources in *Alnus firma* Grown in Yeosu Industrial Complex(I)

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# 여수산업단지에서 자라는 사방오리나무에서 채취한 종자와 질소고정 뿌리혹의 접종 효과 비교(I)

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**ABSTRACT**

**This study identified the physiological characteristics of *Alnus firma* seedlings collected from air polluted industrial complex of Yeosu area with inoculation of nitrogen-fixing nodule. A. *firma* individuals resistant or sensitive to air pollution stress at the study area were selected. Seeds of the individuals were sown in a pot and inoculated with the nodules collected at the same area about 3 weeks after germination. To compare the inoculation effects, photosynthesis was measured among the *A. firma* seedlings under 12 inoculation combinations of seed sources and nodule sources. Photosynthetic activities of seedlings from resistant mother trees were obviously higher than those from sensitive mother trees. In general, the seedlings inoculated with nodule of sensitive trees, showed a worse physiological trend on every combination. In this study, inoculation effect was less important factor than seed sources to photosynthetic activities of *A. firma*. This physiological characteristic seemed to be determined by seed sources rather than nodule sources.**

***Key words*** **: Alnus firma, *germination, inoculation, photosynthesis***

## I. INTRODUCTION

Air pollution is one of the major environmental inhibitors of tree growth and development. Air pollution can either alter physiological processes within a tree and appear as an invisible stress or cause other major metabolic effects as a visible injury(Reich, 1987). It is well known that an environmental stress such as air pollution can cause major evolutionary changes within species(Taylor and Johnson. 1994). Air pollution affects the selection process. Pollutions eliminate the most sensitive genotype and enhance survivorship of resistant genotypes(Houston and Stairs, 1973).

Yeosu industrial complex is located in the southern part of Korea and faced on the South sea (Fig. 1). This area includes many chemical companies. The vegetation of this area has been seriously destroyed. Many investigators have surveyed the causes of damage on this industrial complex(Ghim *et al.*, 1999). Even though the source of air pollution is not clearly identified, ozone and $SO_2$ seem to be two main reasons for poor vegetation in this area.

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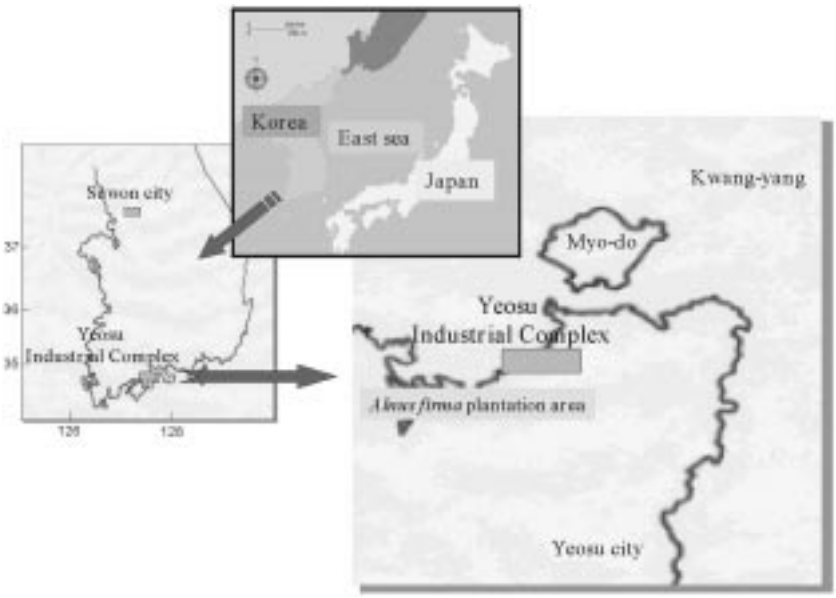


**Fig. 1.** Location of study area.

Among *Alnus* spp., *A. firma* is an important species in natural ecosystem and has potential for use in soil improvement and their contributions to wood and fiber production(Cha and Lee, 1996). *A. firma* occupies temperate regions throughout the world and has shallow root system. Although this species has not been classified to *Leguminaceae*, it has been known to have close relationship with symbiotic nitrogen-fixation microorganisms(Bergersen, 1982). So, *A. firma* can grow extremely harsh environment such as air-polluted area, closed mine and even could help to improve soil condition(Binckley *et al*., 1984). Generally, this species shows good growth performance in air-polluted area such as industrial complex and urban area.

The objective of this study was to compare inoculation effects of *A. firma* seedlings collected from different seed sources such as resistant and sensitive *A. firma* mother trees to air pollution in Yeosu industrial complex. The research strategy was to compare the photosynthesis among the alder seedlings with 12 different inoculation combinations.

## II. MATERIALS AND METHODS

### 2.1. Seed collection, plants materials and growth condition

Seeds of *A. firma* were collected at Yeosu industrial complex as a polluted area and Suwon as a non-polluted area (Table 1 and Fig. 1). These seeds were stored at 4°C in a refrigerator until sowing. Vigor seeds were collected and hundreds of seeds were soaked in tap water for 24 hours and surface-sterilized by agitating in 2% sodium hypochlorite solution for 15 minutes. The seeds were rinsed five times with sterilized distilled water and sown in a plastic pot containing sand autoclaved at 125°C for 30 minutes.

Two weeks after germination, they were placed in the greenhouse under artificial light intensity of 1000-1500

**Table 1.** Condition of *A. firma* selected trees in this study as mother trees

| Places | Visible damage | Percentage of air pollution damage (%) | Age (Year) | Height (m) | DBH (cm) |
|---|---|---|---|---|---|
| Yeosu industrial complex (Polluted area) | No visible damage | 0 | 15 | 4.9± 0.6* | 8.9± 2.4 |
| | Necrosis | 20 | 15 | 4.2± 0.8 | 6.7± 1.3 |
| Suwon(Non-polluted area) | No visible damage | 0 | 10 | 4.7± 0.9 | 8.0± 1.2 |

(* indicates standard deviation)

μmol $m^{-2}$ $sec^{-1}$ at 70% relative humidity. Each seedling was planted into in 500 m*l* pots which contained sand only. Pots were watered daily.

### 2.2. Inoculation with crushed nodules

Root nodules of *A. firma* were collected at Yeosu and Suwon at the 5 different mature tree individuals and mixed (Table 1). After root nodules were first washed in distilled water, nodule lobes were sterilized in a flask containing 1.05% sodium hypochlorite by agitating them for 5 minutes and then washed three times with sterilized distilled water and crushed.

The crushed nodules were inoculated into 3 weeks old *A. firma* seedlings. Crushed suspension solution was used to inoculate and 10 m*l* of this solution was spayed into the sand soil for first 3 weeks. Then 5 seedlings from each treatment were selected to measure photosynthesis. These 5 replicated seedlings showed the root nodule on every seedling later on the inoculation. Seedlings were watered and fertilized twice a week.

### 2.3. Inoculation design

Tolerant and sensitive *A. firma* mature individuals at the Yeosu industrial complex were sampled (40 trees for each) based on visible damage such as necrosis (Table 1). All of these trees were growing near the industrial complex. In addition, *A. firma* trees in Suwon as a non-polluted area were selected as mother trees to collect seeds. Percentages of necrosis for the putative tolerant trees were smaller than those for the putative sensitive ones. Average age, height and DBH for selected trees are similar among the three categories.

Seeds and root nodules of *A. firma* were collected from three different mother trees selected as described previously. Root nodules are found in the upper layer of the soil or even on soil surface. In this study, nine different inoculation combinations were adapted to identify the inoculation effects of *A. firma* seedlings germinated from different seed sources in Yeosu industrial complex and Suwon area. Each inoculation combination was replicated five times.

### 2.4. Measurement of photosynthesis

*2.4.1. Net photosynthesis*

Light-saturated net photosynthesis($A_n$) was measured on a recently mature leaf, Leaf Plastachron Index (LPI) 3 or 4 on every individual in the treatments. Net photosynthesis was measured with a broad-leaf cuvette of the Licor-6400 Portable Photosynthesis System (Licor Inc., USA). The leaf was sealed and $CO_2$ concentration was allowed to be maintained at ambient levels; 360 μmol $CO_2$ $mol^{-1}$ air. Air flow through the analyzer was adjusted to maintain leaf cuvette relative humidity near ambient levels(ranged from 60-70%) during measurement. The average cuvette temperature was maintained at 25ºC. Light intensity (Photosynthetically Active Radiation; PAR) was maintained to 1000 μmol $m^{-2}$ $s^{-1}$. Net Photosynthesis was calculated based on the following formula (Li-cor Inc., 1999).

$$A_n = \frac{U_e(C_e - C_c)}{100s} - C_cE$$

$A_n$; Net Photosynthesis(μ mol $CO_2$ $m^{-2}$ $s^{-1}$), $U_e$; mole flow rate of air entering the leaf chamber(μ mol $s^{-1}$), Ce; mole fraction of $CO_2$ in the leaf chamber(μ mol $CO_2$ $mol^{-1}$ air), $C_c$; mole fraction of $CO_2$ entering in the leaf chamber(μ mol $CO_2$ $mol^{-1}$ air), s; leaf area($cm^2$), E; transpiration(mmol $H_2O$ $m^{-2}$ $s^{-1}$)

### 2.5. Data analysis

Analysis of variance was used to estimate significance of treatment effects. Duncan's multiple range test was used to separate treatment means. All statistical analyses were conducted using SPSS PC+ (version 4.0).

## III. RESULT

### 3.1. Germination rate

The germination rate of *A. firma* in this study ranged from 44.8 to 46.7% (Table 2). *A. firma* seeds from different seed sources showed similar germination rates. There was no effect of mother tree on germination rate.

**Table 2.** Germination rate of *A. firma* from different seed sources

| Species | Seed collection places | Condition of mother trees | Germination rate(%) |
|---|---|---|---|
| *A. firma* | Suwon (Non-polluted area) | No visible damage | 46.7 |
| | Yeosu industrial complex(Polluted area) | No visible damage(Resistant trees) | 46.1 |
| | Yeosu industrial complex(Polluted area) | Visible damage(Sensitive trees) | 44.8 |

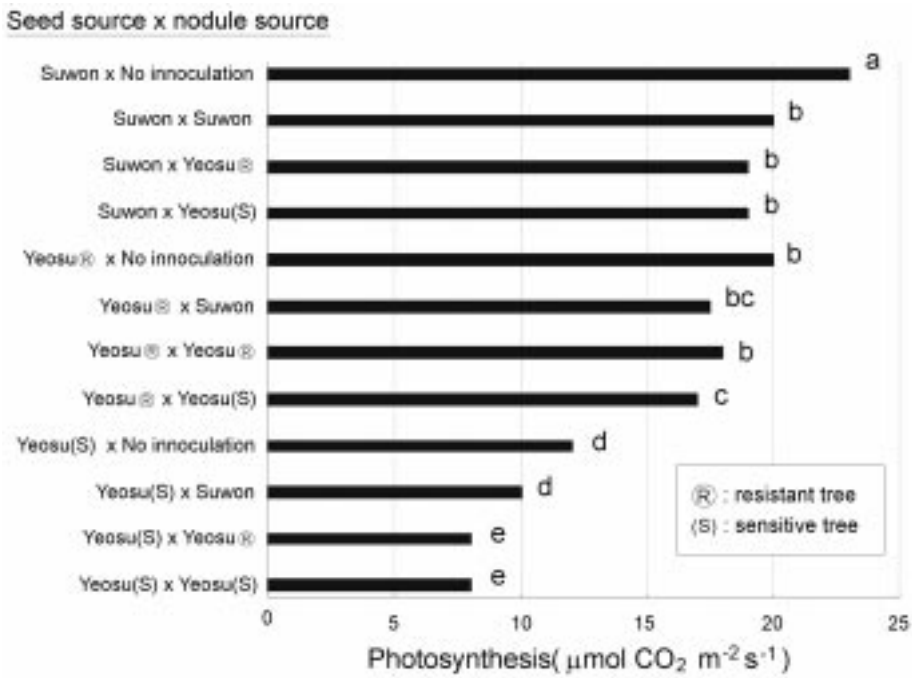


**Fig. 2.** Photosynthetic activity of *A. firma* seedlings. Bars followed by different letters were statistically different at the 5% significant level(n=5).

### 3.2. Photosynthesis

Both seed source (Suwon vs. Yeosu) and mother tree pollution resistance had a significant effect on seedling light-saturated net photosynthetic rates at the 5% significance level (Fig. 2). Light-saturated net photosynthetic rates of seedlings from Suwon and resistant mother trees in Yeosu industrial complex were significantly higher than those from sensitive mother trees.

In the view of inoculation combinations between seed and nodule sources, photosynthetic activity of the seedlings at the seed sources from Suwon did not show a significant reduction in that of seedlings inoculated with any nodule sources. For seedlings from resistant tree, photosynthetic activity of seedlings inoculated with nodules of resistant tree was significantly higher than that of nodule of sensitive tree. In addition, seed of sensitive tree and nodule from Yeosu industrial complex inoculation combination showed the lowest photosynthetic activities (Fig. 2).

## IV. DISCUSSION

### 4.1. Germination rates

Generally, germination rates of *A. firma* were higher than other alders such as *A. hirsuta*, *A. japonica* and *A. glutinosa*(Lim, 1988). The germination rates of *A. hirsuta*, *A. japonica* and *A. glutinosa* in Lim(1988) were 15.5%, 1.2% and 3.5% respectively. The seed in Lim(1988)'s report were collected in Suwon, as in the present study. The highest germination rate was also reported in *A. firma* among other alders.

Germination rate seems to be not related to condition of mother tree because the seed were collected from sensitive mother trees showed similar germination rate to that from resistant trees (Table 2). Germination rate is probably more determined by size of seeds and germination environments such as temperature and moisture rather than the condition of the mother tree (Kozlowski, 1972).

### 4.2. Photosynthesis

Seedlings of resistant trees showed higher photosynthetic activity than those of sensitive trees (Fig. 2). In the same study area, mature *Alnus firma* that showed resistance to air pollution showed significantly higher photosynthetic activity than those of sensitive trees (Woo and Lee, 1999). This result is similar to that reported by Sheng *et al.*(1997) who observed that

photosynthetic activities in air pollution-resistant clones was significantly greater than in sensitive clone for trembling aspen(*Populus tremuloides*).

These results suggest that the polluted conditions at the Yeosu complex may be creating selection pressure for higher photosynthetic and stomatal conductance rates, as well as elevated chlorophyll contents. Stressful environments such as air pollution induce many variations in tree physiological processes during evolutionary processes(Houston and Stairs, 1973). This fact was supported by that several genetic characteristics of species such as *Prunus sargentii* revealed that a greater amount of genetic variations existed in the tolerant trees to air pollution(Lee *et al*., 1998).

Photosynthetic activity seemed to be determined by seed sources rather than nodule sources. Trends of reduction in photosynthetic activity on three seed sources; Suwon, resistant and sensitive trees demonstrated similar reduction when seedlings were inoculated with their nodules of three mother tree counterparts (Fig. 2). This fact indicated that the regulation of photosynthetic activity under various inoculation combinations with the nodules of different mother trees was less important than what seed source was. It has been shown by cross-inoculation studies using *Frankia* cultures that the capacity of seedlings is entirely determined by the characteristics in seeds of mother trees(Burggraaf *et al*., 1983).

## V. CONCLUSION

Photosynthetic activities of seedlings from resistant mother trees were higher than those from pollution-sensitive mother trees. Photosynthetic activity of the Suwon seed did not show a significant reduction in that of seedlings inoculated with any nodule sources. In general, the seedlings inoculated with nodule of sensitive trees, showed a low physiological trend on every combination. In this study, inoculation effect was less important factor than seed sources to photosynthetic activities of *A. firma*. These physiological characteristics seemed to be determined by seed sources rather than nodule sources.

## 적 요

여수산업단지 같은 대기오염지역에서 채취한 사방오리나무의 질소고정 뿌리혹의 접종 효과를 보기 위해서 오염에 대해서 저항성 개체와 민감한 개체를 선발하고 종자를 채취한 후 파종을 하고 발아한 후 3주 후의 유묘를 같은 지역에서 채취한 뿌리혹으로 12개의 조합으로 접종을 하였다. 접종효과를 비교하기 위해서 광합성 능력을 측정하였다.

대기오염에 대해 저항성을 보이는 모수에서 채취한 종자가 민감한 개체에서 채취한 것보다 광합성 능력이 높은 경향을 나타냈다. 대기오염에 민감한 모수에서 채취한 뿌리혹으로 접종한 조합이 일반적으로 생리활성이 낮았다. 그러나 접종효과는 뿌리혹을 채취한 모수보다는 종자를 채취한 모수가 어떤 상태인가에 따라서 광합성 능력이 더 영향을 많이 받는 것으로 나타났다. 그렇기 때문에 생리적인 특성에 대한 영향은 뿌리혹을 채취한 모수보다는 종자의 모수가 더 중요한 요인 같다.

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