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Adaptation of *Betula schmidtii* Seedling in Coal-mine Field with Different Sewage Sludge Treatment Methods

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ABSTRACT: We tested the field adaptation of *Betula schmidtii* on the abandoned coal-mine soil with sludge amendment methods for promoting physiological activity of *B. schmidtii* seedlings under several environmental stress. Sewage sludges were amended to coal-mine soil with *B. schmidtii* seedlings which grown in the mixture of artificial soil and composted sludge soil before transplanting (before-fertilized treatment, BF) and fertilized with composted sludge after transplanting (after-fertilized treatment, AF). The percent of establishment of seedlings for AF (80.7%) was lower than that for BF (92.7%). Nitrate reductase activity and photosynthetic pigment content were higher in AF than in BF, but malondialdehyde (MDA) content and superoxide dismutase (SOD) activity were lower in AF than in BF. These results represent that after-fertilized seedlings increase resistance against physiological stress at field condition using nitrogen source of composted sludge. On the contrary, before-fertilized seedlings were susceptible to environmental stress on abandoned coal-mine soil by exhausting of nitrogen source from composted sludge.

Key words: Coal-mine soil, Malondialdehyde, Nitrate reductase activity, Photosynthetic pigment, Sewage sludge, Superoxide dismutase

INTRODUCTION

In Korea, there are many abandoned mine fields, which has caused environmental and social problems, including ground pollution, run-off, soil erosion, landslides, sodicification and desertification, unemployment, and severe conflicts between the farming and mining industries (Hu 2000). To solve these problems, the Coal Industry Promotion Board (CIPB) has conducted rehabilitation and revegetation projects on abandoned coal-mine lands at government expense.

As a results, it seemed to be successful in early forestation phase but it have been faced the limitation to forestation with poor soil conditions. So, we need to develope the new techniques for effective reforestation of tailings in harmony with neighboring environment.

In general, coal-mine tailings have low organic contents, lacking of nutrients, coarse texture, poor buffer capacity, low water holding ability (Hossner and Hons 1992). And physical, nutritional, and biological characteristics in tailings slow down the plant growth as stress factors which caused the delay of natural succession (Jha and Singh 1992, Logan 1992). Therefore, for a successful revegetation in these futile, it is desirable for improvements in soil's physicochemical properties and selection of highly adaptable species in tailings.

Recently many countries have developed environmental friendly and ecological restoration technology for the revegetation of these disturbed area. For the purpose, they investigated indigenous plants in abandoned coal-mine area or on tailings, and then have used for revegetation the selected species which had best field adaptation (Kim *et al*. 2000, Lee *et al*. 2002, Dutta and Agrawal 2003). We reported that *B. schmidtii* was a pioneer tree species on the abandoned coal-mine lands and a highly adaptable species in poor tailing area (Lee *et al*. 2003b).

Sludge amendments were developed for improvement the soil conditions in poor tailings and increase the plant productivity with recycling of sewage sludge which has various organics(Yum *et al*. 1999). Sludge amendments with recycling of sewage sludge increase the plant growth and prevent the proliferation of various pollutant in tailings (Caravaca *et al*. 2003, Han *et al*. 2004). Because most of these researches were run on the small scale in laboratory simulated conditions, it was hard to find out the adverse effects when it was exposed on abandoned coal-mine field with various environmental factors.

Based on the various results from lab experiments, we tried to test the adaptability of *B. schmidtii* on abandoned coal-mine field where had various stresses factors. We also tried to investigate the fertilization methods to increase the physiological activities of planted *B. schmidtii* at tailings.

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MATERIALS AND METHODS

Plant Material and Soil Preparation

Betula schmidtii is a pioneer tree species on abandoned coal-mine lands located at the Sododong in the Taebaek area of Korea (Lee *et al*. 2002). We collected seeds of this species from a wild population growing in tailings on this site. The seeds were sown in containers with vermiculite in early Spring 2002. Each container was irrigated immediately after sowing and then at two-week intervals.

We choose total 450 individuals of *B. schmidtii* seedlings with constant height, and then divided three groups. Among these, two groups transplanted in plastic basins with artificial soil, which consisted of 1:1:1 sand: peat moss: vermiculite (volume basis), and the remainder group transplanted in mixed soil with 75% of artificial soil and 25% of composted sewage sludge from the Taebaek treatment plant. Sewage sludge was composted with several additives for approximately 45 days. One year later, average height of the one was 16.1 cm and the other was 41.2 cm.

Construction of Field Testing Forest

We constructed field testing forest in abandoned coal-mine area of Jeongsun-gun Kangwon Province (W 128°52′833″, N 37°11′863″) on May, 2003. Field test design was consisted of control group without composted sludge, fertilized with composted sludge after transplanting (after-fertilized treatment, AF), and composted sludge soil before transplanting (before-fertilized treatment, BF).

Also we divided three block to minimize the effect of slope and soil properties, and planted fifty individuals per group in one block. Average temperature and relative humidity of this area from 2003 to 2004 were 9.2℃ and 67% and annual total precipitation was 1,560 mm.

Physiological Activity

We investigated establishment percent and collected leaf sample in order to measure physiological activity of seedling according to sludge-amended methods on August 2004. We analyzed nitrate reductase (NR) activity, chlorophyll content, malondialdehyde (MDA) content and superoxide dismutase (SOD) activity in leaves. We measured NR activity with slightly modified Högberg *et al*. method (1986), chlorophyll content followed Hiscox and Israelstam method (1979). MDA content as damaged level in leaves determined by the method of Esterbauer and Cheeseman (1990), SOD activity was analyzed according to the nitroblue tetrazolium (NBT)-xanthine oxidase method (Beauchamp and Fridovich 1971).

RESULTS AND DISCUSSION

Establishment Percent

To evaluate the field adaptability, we investigated the establishment percent of *B. schmidtii* seedlings among control, before-fertilized and after-fertilized soil. Before-fertilized seedlings showed the highest establishment percent as 92.7% compared with 80.7% for after-fertilized seedlings and 72.7% for control seedlings (Fig. 1).

This result represents that the fertilization methods affect the early establishment rate of tree and activity of seedlings, and the viability of seedlings highly depends on the nutritional state of soil when they were transplanted. In particularly, before-fertilized soil provided the enough nutrition to the seedlings to grow up actively, which resulted in increased field adaption with improving the physiological activity (Caravaca *et al*. 2002, Han *et al*. 2004). However, after-fertilized seedlings showed poor establishment rate than the before-fertilized seedlings, which might come from insufficient nutritional supply in root. It is likely that the composted sludge provided the dissolved nutrient to the soil but was not enough time to increase the activity in root system.

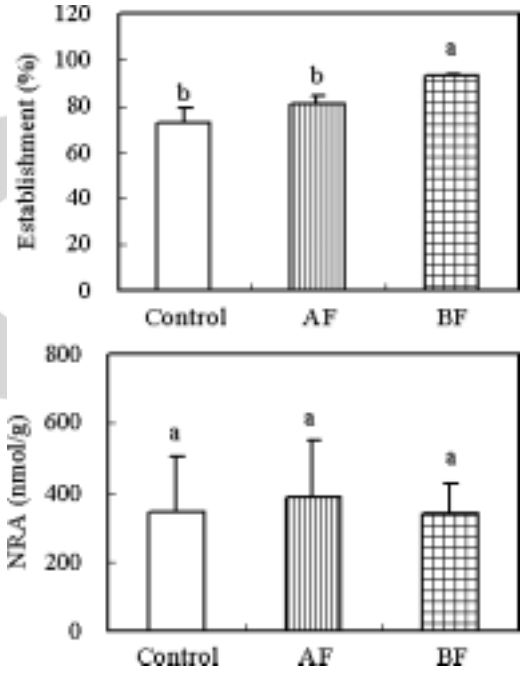

Fig. 1. Establishment percent and nitrate reductase activities (NRA) in the leaves of *B. schmidtii* seedlings in response to composted sludge addition on abandoned mine soils. AF and BF indicate seedlings which fertilized with composted sludge after transplanting on abandoned mine soil and grown in the mixture of artificial soil, which consisted of 1:1:1 sand : peat moss : vermiculite (volume basis), and composted sludge soil before transplanting on abandoned mine soil, respectively. Means with the same letter are not significantly different at the 5% probability level by Duncan's multiple range test.

Nitrate Reductase Activity

Nitrate reductase activity among sludge-amended methods was highest for after-fertilized seedlings as 383 nmol/g compared with 341 nmol/g for before-fertilized seedlings and 346 nmol/g for control (Fig. 1). However, there was no statistically significant different as shown in establishment rate. It is because the unevenness in soil quality caused the difference between block (Pr > F, 0.0001).

Högberg *et al*. (1986) applied nitrate reductase activity in leaf as indirect indicator for the nitrogen use in soil. In this study, after-fertilized seedlings showed the highest nitrogen reductase activity which implied that the nitrogen in sludge was dissolved in soil and then nitrogen was used in plant metabolism. However, before-fertilized seedlings showed poor nitrate reductase activity as low as control which came from the consumption of nitrogen in soil by plant growth and elution of nitrogen nutrient in irrigation. Therefore we recommend periodical fertilization and management to keep up the physiological activity of planted tree in field.

Chlorophyll and Carotenoid Content

After-fertilized seedlings showed the highest total chlorophyll content in leaf as 2.01 mg/g compared with 1.89 mg/g for before-fertilized and control seedlings. In carotenoid content, after-fertilized seedlings also showed the highest content as 0.41 mg/g compared with 0.38 mg/g for before-fertilized and control seedlings (Table 1). Ratio of chlorophyll a and b as 1.74 for after-fertilized seedlings and the ratio of total chlorophyll content and carotenoid was 4.82.

In Table 1, after-fertilized seedlings showed the highest total chlorophyll content which came from the higher chlorophyll a content than the other treatment or control, which made higher ratio of chlorophyll a and b than others. In general, the ratio of chlorophyll a and b is 1~3 range, but this ratio was decreased because chlorophyll a was damaged easier than chlorophyll b in environmental stresses (Sane *et al*. 1996, Lee *et al*. 2003a).

In addition, after-fertilized seedlings showed the higher carotenoid content than others, which resulted in lower ratio of total chlorophyll content to carotenoid content. The effect of nitrogen nutrient supply from sludge was the reason for higher chlorophyll and carotenoid content after fertilizing (Han *et al*. 2004), and higher nitrate reductase activity proved that nitrogen from sludge was used in planted tree. However, nitrogen nutrients was sharply decreased in before-fertilized seedlings and showed similar level of chlorophyll content with control.

MDA Content

There was significant difference in MDA content in leaf (Fig. 2). In treatment, before-fertilized seedlings showed the highest MDA content as 619 nmol/g, for after-fertilized, MDA content was 479 nmol/g which was lower than control. Stress-exposed plant could get damage in cell membrane otherwise the active oxygen species were completely removed in plant metabolism (Davis and Swanson 2001). In this study, before-fertilized one showed higher establishment percent with prosperous growth in pot but with the depletion of nitrogen nutrients in field resulted in higher MDA content than control with higher tissue damage from exposure of various stress factors in field. Increase of MDA content in leaf also was observed in leaf of indigenous *B. schmidtii* under various stress factors on tailings (Lee *et al*. 2003b).

SOD Activity

After-fertilized seedlings gave the lowest SOD activity in leaf as 897 unit/g, before-fertilized seedlings showed the highest as 1,174 unit/g (Fig. 2). Like this, artificial soil with sludge showed higher SOD activity as shown similar results in nitrate reductase activity, the similar reason for higher nitrate reductase activity in after-fertilized. These results also related with higher chlorophyll and carotenoid content in after-fertilized than others, nitrogen in sludge afford to make enough carotenoid for antioxidant activity. Nitrogen in sludge could improve the resistance toward stress with increasing the physiological activity in tree (Han *et al*. 2004).

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Table 1. Photosynthetic pigment content in the leaves of *B. schmidtii* seedlings in response to composted sludge addition on abandoned mine soils.

Treatment	Ca	Cb	Ca+b	Car	Ca/b	Chl/Car
	mg/g					
Control	1.08±0.17^{b}	0.69±0.17^{a}	1.77±0.31^{a}	0.35±0.03^{b}	1.60±0.35^{a}	5.04±0.64^{a}
AF	1.24±0.15^{a}	0.71±0.21^{a}	2.01±0.32^{a}	0.41±0.06^{a}	1.74±0.43^{a}	4.82±0.77^{a}
BF	1.15±0.25^{ab}	0.72±0.21^{a}	1.89±0.41^{a}	0.38±0.06^{ab}	1.65±0.36^{a}	4.91±0.79^{a}

Ca, chlorophyll a; Cb, chlorophyll b; Ca+b, total chlorophyll; Car, total carotenoid; Ca/b, the ratio of chlorophyll a and b; Chl/Car, the ratio of total chlorophyll and carotenoid. Means with the same letter are not significantly different at the 5% probability level by Duncan's multiple range test.

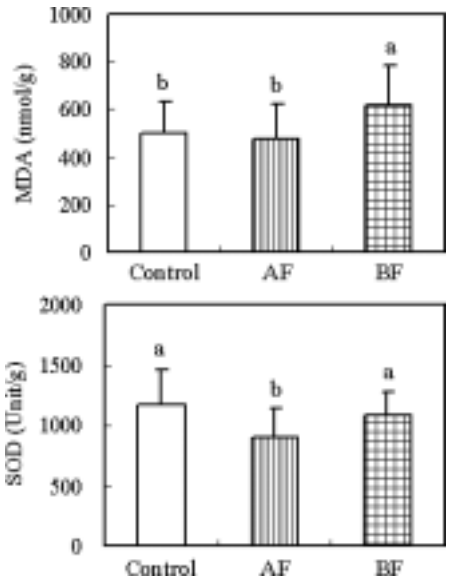

Fig. 2. MDA contents and SOD activities in the leaves of *B. schmidtii* seedlings in response to composted sludge addition on abandoned mine soils. Means with the same letter are not significantly different at the 5% probability level by Duncan's multiple range test.

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