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Full Length Research Paper

Epidermal leaf characteristics and seasonal changes of net photosynthesis of five *Populus*

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Net photosynthesis (P_N) and leaf epidermal characteristics of five poplars; 72-31 (*Populus alba x Populus glandulosa*), Eco28 (*Populus euramericana*), Suwon (*Populus koreana x Populus nigra* var. *italica*), Dorskamp (*Populus deltoides x P. nigra*), and 62-2 (*P. nigra x Populus maximowiczii*) were investigated to come up with parameters for comparison. The poplars, 72-31, Suwon and Dorskamp had a significant P_N decreasing from May through September. A wide significant variation in the stomata size and density of the genotypes were observed. Especially, Dorskamp and 62-2 had significantly lower number of stomata than Eco-28, 72-31 and Suwon had, but significantly larger stomata length than Suwon and 72-31 had. In addition, 72-31 had trichomes in the abaxial surface, which may be one of the phytoremediation characteristics of this clone compared to other clones.

Key words: Leaf epidermis, net photosynthesis, *Populus*, stomata, trichome.

INTRODUCTION

Populus sp. is widely distributed over the Northern hemisphere (Stettler et al., 1996). In recent years, poplars have been receiving increasing attention for phytoremedition. Fast-growing species such as *Populus* can absorb toxic materials through their roots and transport them to their stems or leaves (Kukaszewiski et al., 1993; Meloni et al., 2003). Fast-growing species are used in Korea for phytoremediation. There are four native poplar species in South Korea: *Populus glandulosa*, *Populus davidiana*, *Populus simonii*, and *Populus maximowiczii*. *P. glandulosa* and *P. davidiana* are naturally distributed along the eastern mountain range of the South Korea. *P. simonii* grows in the central Korea, and *P. maximowiczii* is naturally distributed along the creeks in the eastern mountainous region.

Due to the limited *Populus* species in Korea, hybridization has been supported by the Korean government. However, differences in the physiological and epidermal characteristics of several poplar clones have not been carefully investigated. The objective of this study was to compare epidermal characteristics and seasonal net photosynthesis changes of five poplar clones.

Nomenclatures: P_N, Net photosynthesis; 72-31, *Populus alba x Populus glandulosa*; Eco28, *Populus euramericana*; Suwon, *Populus koreana x Populus nigra* var. *italic*; Dorskamp, *Populus deltoides x P. nigra*; 62-2, *P. nigra x Populus maximowiczii*; **LPI,** leaf plastachron index

MATERIALS AND METHODS

Plant materials

Five poplar clones were collected as 20 cm cuttings from the Poplar Farm nursery in Suwon. Each cutting was dipped into 100 µg/g of Indole-3-butyric acid, and then planted in pots containing peat, vermiculite and soil at a 1:1:1 (v/v/v) ratio. Each clone had five replicates. The plants were irrigated using tab water (3 l/day). The temperature in the greenhouse was 23 - 25℃ and the relative humidity reached 60-80% at the seedling height. The midday photosynthetic photon flux density ranged from 700 to 1,000 µmol /m^2/s.

Five poplar clones - 72-31 (*Populus alba x P. glandulosa*), Eco28 (*Populus euramericana*), Suwon (*P. koreana x Populus nigra* var. *italica*), Dorskamp (*P. deltoides x P. nigra*) and 62-2 (*P. nigra x P. maximowiczii*) - were used and the experiment plots were set in the greenhouse. Five replications were applied for each poplar (n = 5 per clone).

Net photosynthesis

The P_N was measured on a single leaf and leaf plastachron index (LPI) 5 on every individual. The P_N was measured with a broad-leaf

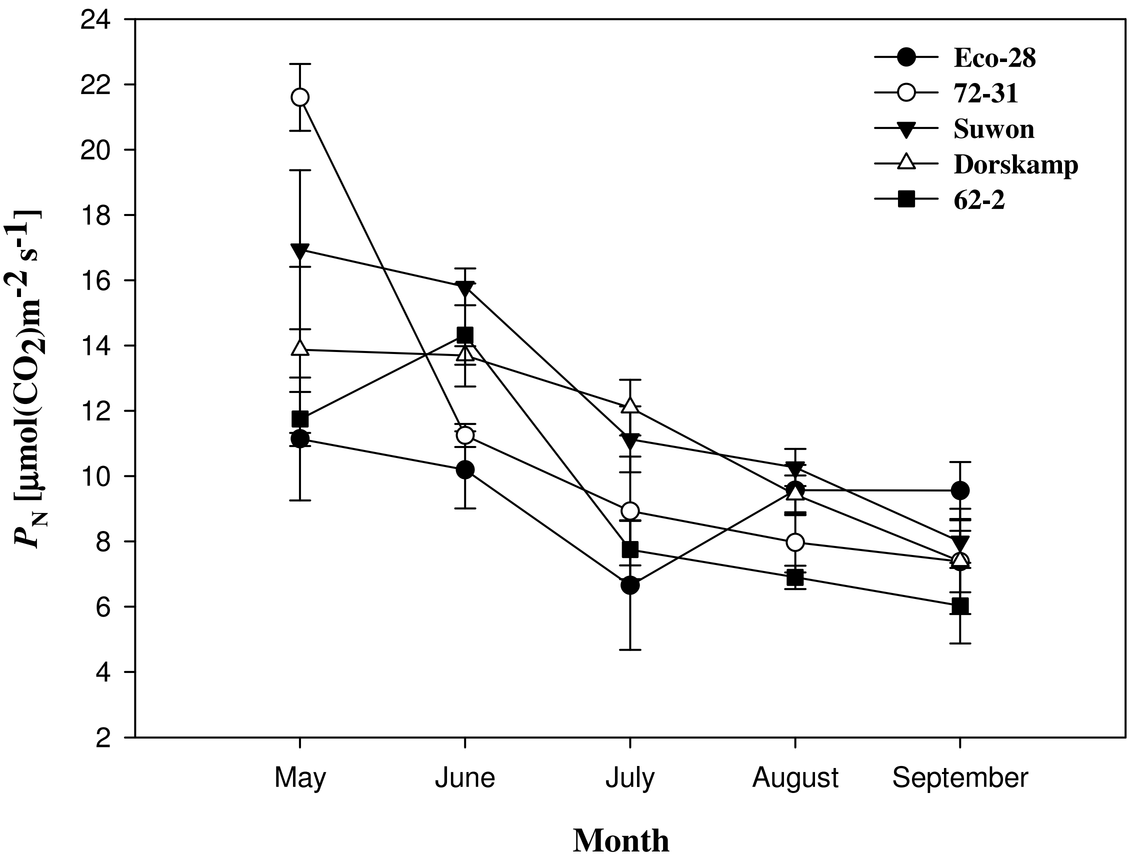

Figure 1. Seasonal changes in the net photosynthetic rate (P_N) of five poplar clones. Bars indicate standard deviation (n = 5).

cuvette from the Licor-6400 Portable Photosynthesis System (Licor Lincoln, NE, USA). The leaf was sealed and the CO_2 concentration was maintained at 400 µmol /mol CO_2 levels. Five replications were done for each tree. Differences in the seasonal P_N were averaged for all measurements of each replicate, and the standard deviations were compared.

Scanning electron microscope (SEM)

The leaf abbatial surface and the stomata were investigated using a scanning electronic microscope (SEM-JSM-5410LV, JEOL, Tokyo, Japan) to find out the stoma characteristics of each poplar. Fresh leaves were carefully pressed into the dental latex. The samples were coated with gold/palladium in a coater and examined under a SEM (Soukuopvá et al., 2000). Measurements of stomata were determined in the SEM equipped with a micrometer. For the width of stomata, both guard cells were measured including the stomatal slit.

Statistical analysis

Results are expressed as the averages and standard deviations of every measurement. Mean values of stomatal densities and size on abaxial surfaces of each poplar were analyzed by Statistical Package for the Social Sciences (SPSS) statistical package (Version 13). One-way ANOVA with a general linear model (GLM) was used and least significant difference (LSD; α = 5%) values were used to compare means.

RESULTS AND DISCUSSION

Net photosynthesis (P_N)

The poplar clones demonstrated a general decrease in P_N from May through September, except clones Eco-28 and 62-2 (Figure 1). Especially, the P_N of clones 72-31, Suwon and Dorskamp were down-regulated from May through September. The P_N in May was higher than that in July, August and September. The fast-growing species generally showed very high growth parameters such as P_N, transpiration rates and stomatal openness during the leaf expansion season in May and June. All poplar clones showed highest photosynthetic rates in May, except clone 62-2.

The P_N of the leaves varied significantly during their development (Kozlowski and Parllardy, 1997). In general, for most of the fast-growing species such as poplar and willow which produce leaves during much of the growing season, the P_N of the individual leaves declined rapidly after the maximum rate was reached near the time of full leaf expansion. The P_N of the leaves increased to the

Table 1. Mean values of stomatal densities lengths and widths on abaxial surfaces of each poplar.

Poplar clones	Density (100 x 100 μm)	Size (μm)	
		Length (μm)	Width (μm)
Eco-28	6.20 ± 1.48 [b]	2.27 ± 0.24 [a]	1.13 ± 0.09 [ab]
72-31	6.60 ± 0.55 [a]	1.88 ± 0.34 [b]	0.99 ± 0.21 [b]
Suwon	6.20 ± 1.48 [b]	2.19 ± 0.07 [b]	1.19 ± 0.08 [ab]
Dorskamp	3.60 ± 0.55 [c]	2.45 ± 0.20 [a]	1.16 ± 0.13 [ab]
62-2	3.60 ± 0.55 [c]	2.40 ± 0.26 [a]	1.23 ± 0.22 [a]

Values followed by the same letter are not significantly different among clones at $P < 0.05$ according to LSD tests. Numbers indicate mean ± standard deviation of five replications.

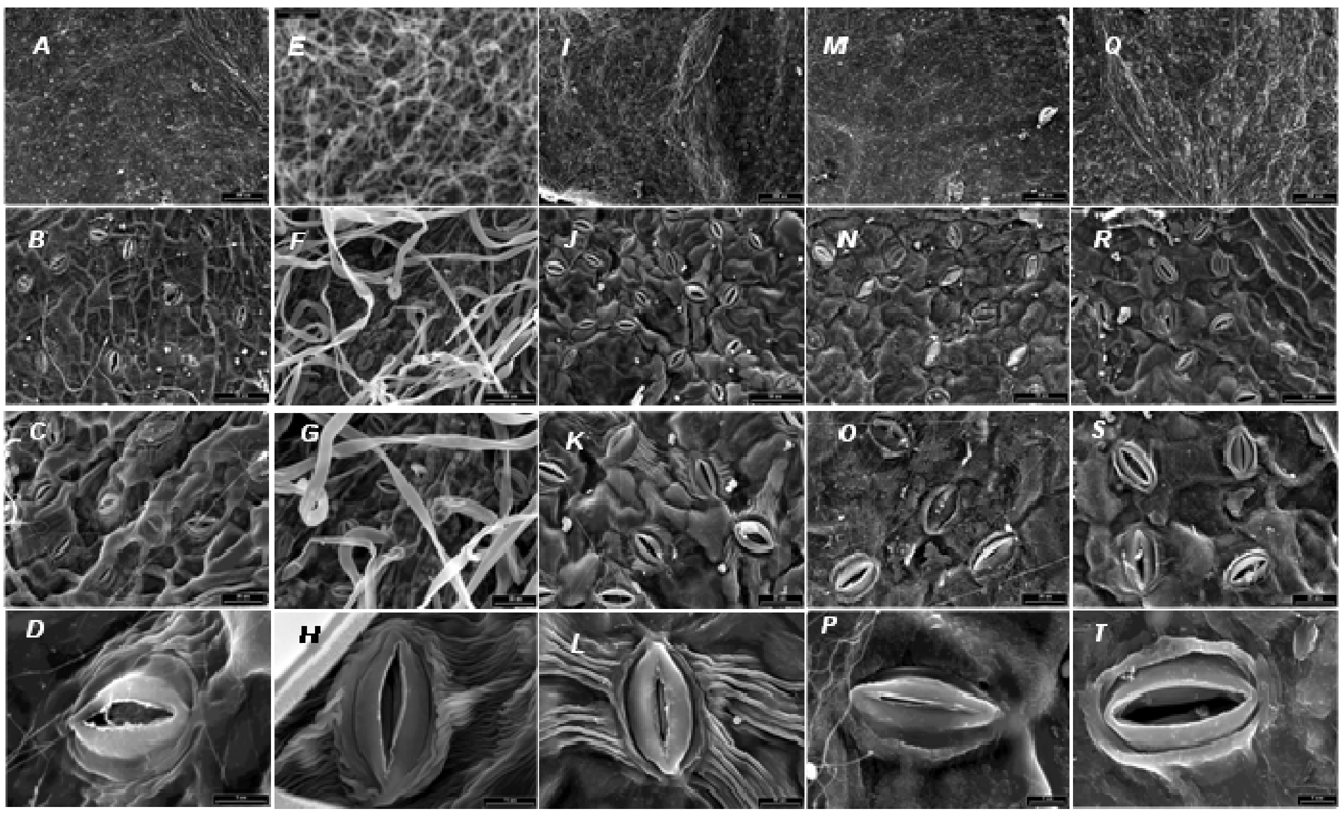

Figure 2. Comparison of scanning electronic microscope (SEM) images of leaves of five poplar clones grown in a greenhouse based on four different scales. A, B, C and D = Eco28 (*P. euramericana*); E, F, G and H = 72-31 (*P. alba x P. glandulosa*,); I, J, K and L = Suwon (*P. koreana x P. nigra* var. *italica*,); M, N, O and P = Dorskamp (*P. deltoides x P. nigra*,); and Q, R, S and T = 62-2 (*P. nigra x P. maximowiczii*).

maximum where the leaves were fully grown and then declined as the leaves senesced.

Stomata density and size

Stomata are among the most important physiological features of the body of a tree (Gravano et al., 2003). Generally, a clone with few stomata per unit of leaf surface tends to have large stomata (Table 1). In this study, Dorskamp and 62-2 had significantly lower number of stomata and larger stomata than Eco-28, 72-31 and Suwon had (Table 1 and Figure 2). Kozlowski and Pallardy (1997) also reported the wide variations in the stomata size and frequency among species and genotypes: *P. maximowiczii* x *P. nigra*, *A. negundo* and *Acer saccharium* had many small stomata and *Populus deltoids*, *P. nigra*, and *Ginkgo biloba* had few but large stomata.

In this study, all the leaves displayed a typical epidermal structure (Figure 2). In fact, their stomata were surrounded by guard cells. Among the five examined poplars, 72-31 (*P. alba x P. glandulosa*) showed many trichomes (Figure 2E, F, G and H). This is the most distinguishable characteristics of this clone. Number of trichomes is a very important feature in terms of environmental aspects such as phytoremediation. This clone can increase the ability of the leaves to retain fine atmospheric particles (Moreno et al., 2003). The surface, the texture of the leaf and the capacity of the stomata to absorb pollutants seem to be the main factors in alleviating mass air pollutants (Robinson et al., 1998; Karnosky et al., 2005; Woo and Hinckley, 2005). Numerous studies have shown that some species such as *Platanus* sp. have hairy leaf surfaces on their abaxial epidermis that can accumulate dust and other pollutants for air pollution reduction.

Poplar clones 72-31 (Figure 2H) and Suwon (*P. koreana x P. nigra* var. *italica*) showed well-developed cuticular ridges (Figure 2K and L). In comparison, the other clones near the guard cells were surrounded only by lipid and wax (Figure 2P and T). This structure seems to be a trigger when a poplar clone faces environmental stresses such as air pollution (Reich et al., 1983; Noormets et al., 2001; Neto et al., 2006). Generally, the cuticular cells near the stomata and the guard cells surrounded by lipid and the wax of the tree species increase the cell wall thickness in the vein in the presence of air pollution (Reig-Armiñana et al., 2004). The stomata surrounded by lipid and wax show a more swollen cell wall and fewer cytoplasmic contents compared to the stomata with well-developed cuticular ridges. The swollen cell wall may squeeze the guard cell, and then induce the closure of the stomata, resulting in the reduction of air pollution penetration into the stomata (Lawson et al., 2002).

In summary, poplar clones demonstrated a general decrease in photosynthetic rates from May through September, except for clones Eco-28 and 62-2. The number of stomata in Dorskamp and 62-2 were significantly fewer than those of three other poplar clones, whereas lengths of stomata were significantly larger compared to 72-31 and Suwon. Clones 72-31 had many trichomes and may be one of the phytoremediation characteristics of this clone.

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