

African Journal of Biotechnology Vol. 9(10), pp. 1455-1458, 8 March, 2010  
 Available online at <http://www.academicjournals.org/AJB>  
 ISSN 1684-5315 © 2010 Academic Journals

## Full Length Research Paper

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

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Accepted 18 January, 2010

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 phytoremediation. Fast-growing species such as *Populus* can absorb toxic materials through their roots and transport them to their stems or leaves (Kukaszewski 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.

## MATERIALS AND METHODS

### Plant materials

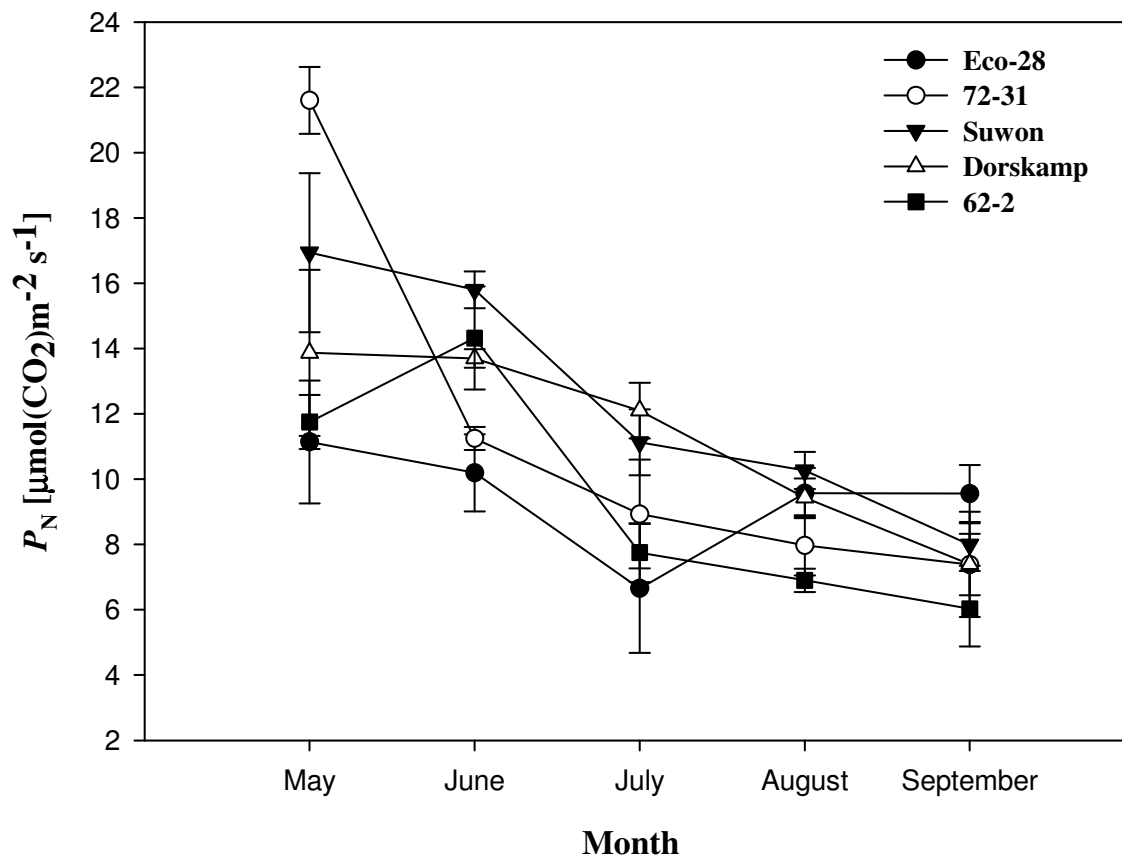
Five poplar clones were collected as 20 cm cuttings from the Poplar Farm nursery in Suwon. Each cutting was dipped into 100 µg/g of Indole-3-butyric acid, and then planted in pots containing peat, vermiculite and soil at a 1:1:1 (v/v/v) ratio. Each clone had five replicates. The plants were irrigated using tap water (3 l/day). The temperature in the greenhouse was 23 - 25°C 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<sup>2</sup>/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 plastichron index (LPI) 5 on every individual. The  $P_N$  was measured with a broad-leaf

**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 plastichron index



**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  $\text{CO}_2$  concentration was maintained at  $400 \mu\text{mol/mol}$   $\text{CO}_2$  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 abaxial 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;  $\alpha = 5\%$ ) values were used to

compare means.

## RESULTS AND DISCUSSION

### Net photosynthesis ( $P_N$ )

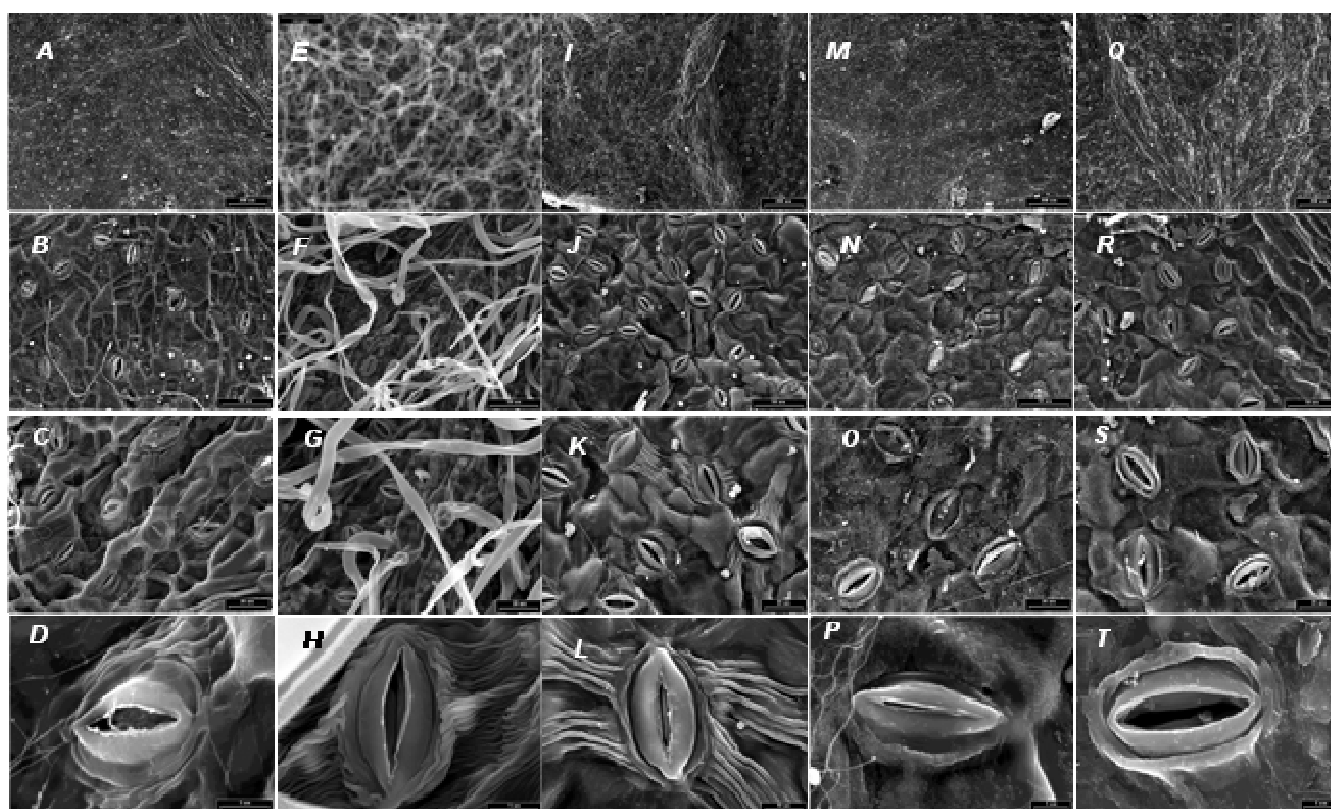
The poplar clones demonstrated a general decrease in  $P_N$  from May through September, except clones Eco-28 and 62-2 (Figure 1). Especially, the  $P_N$  of clones 72-31, Suwon and Dorskamp were down-regulated from May through September. The  $P_N$  in May was higher than that in July, August and September. 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 (Kozłowski 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 $\mu\text{m}$ )	Size ( $\mu\text{m}$ )	
		Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )
Eco-28	6.20 $\pm$ 1.48 <sup>b</sup>	2.27 $\pm$ 0.24 <sup>a</sup>	1.13 $\pm$ 0.09 <sup>ab</sup>
72-31	6.60 $\pm$ 0.55 <sup>a</sup>	1.88 $\pm$ 0.34 <sup>b</sup>	0.99 $\pm$ 0.21 <sup>b</sup>
Suwon	6.20 $\pm$ 1.48 <sup>b</sup>	2.19 $\pm$ 0.07 <sup>b</sup>	1.19 $\pm$ 0.08 <sup>ab</sup>
Dorskamp	3.60 $\pm$ 0.55 <sup>c</sup>	2.45 $\pm$ 0.20 <sup>a</sup>	1.16 $\pm$ 0.13 <sup>ab</sup>
62-2	3.60 $\pm$ 0.55 <sup>c</sup>	2.40 $\pm$ 0.26 <sup>a</sup>	1.23 $\pm$ 0.22 <sup>a</sup>

Values followed by the same letter are not significantly different among clones at  $P < 0.05$  according to LSD tests. Numbers indicate mean  $\pm$  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). Kozłowski 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 deltoides*, *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* × *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* × *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.

## ACKNOWLEDGEMENTS

I would like to thank all my colleagues; Drs. Yeong Bon Koo, Jin Kie Yeo, Kwan Soo Woo and Sun Mi Je of Korea Forest research Institute and Ms. Soo Zin Ryang of the Urban Environment and Plant Science Laboratory of the University of Seoul for their help in data collection and assistance. This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2008-314-F00021).

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