Journal of Bio-Environment Control, 17(2):101~109, (2008)

Effects of Artificial Water Treatment on Chlorophyll Contents and Photosynthetic Characteristics in *Fraxinus rhynchophylla* and *Fraxinus mandshurica* Seedlings

Soo Won Lee[1], Jeong Ho Choi[2*], Jin Chul Chung[3], Kwon Ki Won[4], Se Kuel Yoo[1], and Jong Hyang Bae[5]

[1]*Forest Practice Research Center, Korea Forest Research Institute, Pocheon, 487-821, Korea*
[2]*Resources Development Div. of KENERTEC Co., LTD., Seoul 152-051, Korea*
[3]*Division of Bio-Environment Science, Wonkwang University, Iksan 570-749, Korea*
[4]*Department of Environment and Forest Resources, Chungnam National University, Daejeon 305-764, Korea*
[5]*Division of Horticulture and Pet Animal-Plant Science, Wonkwang University, Iksan 570-749, Korea*

Abstract. The content of chlorophyll a, b have generally increased for *Fraxinus rhynchophylla* and *Fraxinus mandshuricain* the order of month of June < August and < September according to artificial water treatment. As similar tendency to *F. rhynchophylla*, the chlorophyll a content of *F. mandshurica* grown from different treatments of A, B, C and D also demonstrated increment from 0.89 to 1.67 mg/g and the chlorophyll b showed increment from 0.18 to 0.44 mg/g in the order of June < August < September period. Both *F. rhynchophylla* and *F. mandshurica* showed increment in amount of chlorophyll a and b with the lapse of growing period and the increment amount was higher between August and September than that of between June and August. The photosynthetic characteristics that respond to moisture process demonstrated decrease tendency as the moisture condition of the soil is low and as the stress lasted longer. The results showed even more irregular patterns in September. *F. rhynchophylla* that showed higher photosynthetic rate even in September that has past growth time due to the A treatment which has relatively fair soil moisture condition, the B, C and D treatments distracted by stress due to insufficient moisture in the soil are showing relatively lower photosynthetic rate during a comparison of photosynthetic rate by growth stage.

As similar tendency to *F. rhynchophylla*, *F. mandshurica* also demonstrated higher photosynthetic rate according to the treatments in the order of A > B > C and > D. Therefore we suppose the photosynthesis rate will increase if the moisture level is high and in regardless of the growth stage.

Key words : artificial water treatment, chlorophyll contents, *Fraxinus mandshurica*, *Fraxinus rhynchophylla*, photosynthesis characteristic, photosynthetic rate

Introduction

The forest is an organism that reacts to continuous and interactive factors of the environment and clarifying the relationship with the condition of the forest. The inorganic environmental factors is a concrete way to know the growth and development of trees constituting the forest, the enhancement of productivity of the forest, and so forth. Also, the forest forms a unique environment with the crown of forest trees such as the soil water within the forest, light, temperature and humidity that strongly influences the type and appearance rate of understory vegetation, the growth and survival of young trees(Choi, 2001; Freedman, 1989; Lee *et al*, 1997; Lee and Woo, 2000; Kramer and Kozlowski, 1979). Especially, the water gives impact on various inorganic environment along with light within the forest ecosystem and it provides direct and indirect causes inducing the loss of moisture contained in the tree by accelerating the transpiration of leaves. The studies to find out the physiological and ecological impact of water have been made through many researchers in a long term in advanced countries and many results have been reported. The physiological and ecological studies related with moisture and light have been progressed by a few researchers that this were considered as an important

***Corresponding author:** uptake@hanmail.net
Received March 4, 2008; accepted March 14, 2008

task that needs more research efforts(Alberte *et al*, 1977; Ashton and Berlyn, 1994; Bahari *et al*, 1985; Boardman, 1977; Castrillo, 1983; Choe and Lee, 1995; Lee *et al*, 2004; Kramer, 1983; Sim and Han, 2003).

Hence, in order to analyze the special growth characteristics that fluctuate depend on moisture environment, the study focused its analysis on *Fraxinus rhynchophylla* and *Fraxinus mandshurica* which its value is expanding thanks to broad-leaved tree resource.

This study analyzed the impact of artificial water treatment on the chlorophyll contents and photosynthesis by seasonal changes with the seedlings of 2 hardwood species in order to find out the physiological characteristics of each species including water requirements. The purpose of this was to provide basic data required for developing the landscaping and tree, urban forest and forest managing technologies to improve the water tolerance of trees.

Materials and Method

1. Water treatments and soil characteristics

The study was carried out in greenhouse of Wonkwang University, college of natural science during early of April to September 2006.

The amount of moisture variables of growing media container saved in moisture environment process of *F. rhynchophylla* and *F. mandshurica* are as Fig. 1. As a result of irrigation process of 1 day, 3 days, 6 days and 9 days increments, the water content of growing media container was uniformly decreased. The A treatment displayed 78~90% in 1 day increment, 58~70% in B treatment in 3 days increment, 38~50% in C treatment in 6 days increment and 18~30% ranges in D treatment in 9 days increment(Chung *et al*, 2007).

The physical and chemical analysis result on the growing media used for the experiment was as the Table 1. pH, one of the chemical traits of the growing media that combines Vermiculite, Peat Moss and Perlite in 1:1:1 ratio were 6.0 subject to the pH 5.6~6.0(Korea Forest Service, 2000) which was proposed as the broad-leaved tree nursery bed soil. E.C. was 0.1 ds/m, Ca^{++} was 40.0 c·mol^{+}/kg, NO_3 was 0.1 mg/kg, P_2O_5 was 123.0 mg/kg, K^{+} was 4.0 c·mol^{+}/kg, Mg^{++} was 7.0 c·mol^{+}/kg and Cation Exchange Capacity(C.E.C.) was 17.0 c·mol^{+}/kg.

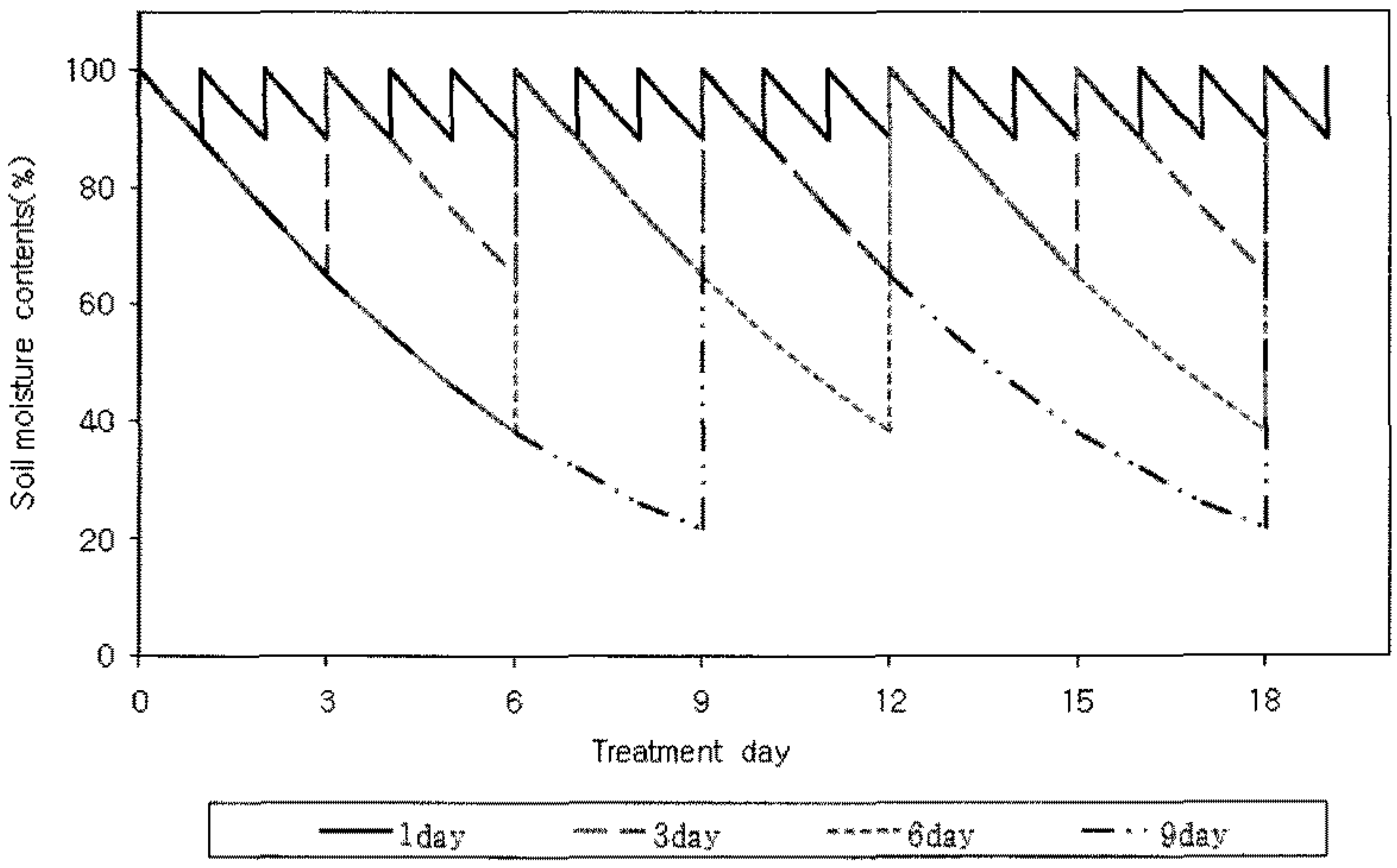

Fig. 1. Daily changes of soil moisture contents in each of dry and wet soil moisture regimes.

Table 1. The physical and chemical properties of the growing media.

Physical elements (%)		Chemical characteristics			
peatmoss	33.0	pH(1 : 5, v/v)	6.0	P_2O_5(mg/kg)	123.0
vermiculite	33.0	E.C(ds/m)	0.1	K^{+}(c·mol^{+}/kg)	4.0
perlite	33.0	Ca^{++}(c·mol^{+}/kg)	40.0	Mg^{++}(c·mol^{+}/kg)	7.0
etc.	1.0	NO_3(mg/kg)	0.1	C.E.C.(c·mol^{+}/kg)	17.0

Table 2. Sachs' of nutrient solution.

Major elements ($g \cdot L^{-1}$)		Minor elements ($g \cdot L^{-1}$)	
KNO_3	1.00	H_3BO_3	0.60
$Ca_3(PO_4)_3$	0.50	$CuSO_4 \cdot 5H_20$	0.05
$MgSO_4 \cdot 7H_2O$	0.50	$MnCl_2 \cdot 4H_2O$	0.40
$CaSO_4$	0.50	$H_2MoO_4 \cdot 4H_2O$	0.02
NaCl	0.25	$ZnSO_4$	0.05
$FeSO_4$	some		

3. Tree materials and analysis

One-year-old *F. rhynchophylla* and *F. mandshurica* seedlings were obtained from Northern Regional Forest Service and transplant the seedling into an ordinary plastic container(15 cm × 18 cm × 12 cm). To exclude growth affect of seedlings in accordance with nutrient, the study used vermiculite, peat moss and perlite of culture soil of early April by mixing them up in a 1 : 1 : 1 ratio as growing media. And to minimize stress accompany by the transplanting, the seedling have been rooted until the early part of June to reinstate their normal physiological condition and selected 80 seedlings respectively for experimental measure. The analysis of used growing media was analyzed by soil chemical analyzing method. The element contained in growing media essentially required for a tree to grow is the minimum. Therefore, the study irrigated nutrient solution of Sachs'(Sachs, 1860) in four weeks increment to supplement essential nutrient needed for growth as the Table 2.

The water treatments were executed in four steps starting from the early part of June in 1 day, 3 days, 6 days and 9 days increments. The soil moisture contents were calculated as following method after drying fixed amount of wet soil in an oven at 100 - 105°C temperature and the measuring was carried out 10 times per each treated area(KFS, 2000).

Soil moisture content (%)
=(Dry soil volume (g)/Raw soil volume (g)) × 100

4. Analysis of chlorophyll contents

To study the artificial water treatments on the chlorophyll contents of 2 tree species of deciduous hardwoods were treated in 4 levels. Tree seedlings were randomly selected for three periods; June which was before water treatment, August when the leaves were maturing during the water treatment and September when the tree's physiological metabolic activities slowed down, and the chlorophyll contents were analyzed 3 times for each seedling, total 9 times, in order to analyze the seasonal changes of chlorophyll contents according to the level of chlorophyll contents according to the level of water treatment. For the extraction of chlorophyll contents, the method using Dimethylsulfoxide (DMSO) conforming to Hiscox and Israelstam(1978) method was applied.

After a fixed period time, the light absorption was measured from the extract inside the test tube, the leaf tissue of which turned light brown, in the wavelength of 663 nm and 645 nm using spectrophotometer and obtained chlorophyll contents a and b using the expression of Arnon(1949) and Mackinney(1941).

Chlorophyll a ($mg \cdot g^{-1} \cdot$ fresh wt.)
$= (0.0127 \times OD663 - 0.00269 \times OD645) \times f$
Chlorophyll b ($mg \cdot g^{-1} \cdot$ fresh wt.)
$= (0.0229 \times OD645 - 0.00468 \times OD663) \times f$
※ OD : Optical Density

In this expression, OD663 and OD645 represent the light absorption at 663 and 645 nm respectively, and f is the function related to the fresh weight and the dilution factor according to DMSO contents.

5. Net photosynthesis measurement

To analyze the photosynthetic characteristics of the experimental trees in relation to water treatment, the time-serial changes such as photosynthetic rate, light saturation point and light compensation point were examined and compared by each species and the level of water treatment using portable photosynthesis measuring device in three periods; June which was before water treatment, mid August when the leaves are maturing during the water treatment, and early September when the growth of the tree slows down.

Light-saturated net photosynthesis(An) was measured on fully expanded, mature leaf number 4 counted from each shoot apex on every individual in the treatments. Net photosynthesis was measured with a broad-leaf cuvette of the Li-cor 6400 Potable Photosynthesis System (Li-cor Inc., USA), the leaf was sealed and CO_2 concentration was allowed to be maintained at ambient levels. Air flow through the analyzer was adjusted to maintain leaf cuvette relative humidity near ambiance level (70±10%) during measurement. The average cuvette temperature was maintained at 25°C. For photosynthesis capacity, this study measured the light-photosynthesis curve by adjusting the light intensity from 0 to 2,000 $\mu mol \cdot m^{-2}s^{-1}$ with a photosynthesis analyzer(Choi, 2001; Woo *et al* a, b, 2004,).

Net Photosynthesis was calculated as :

$$A_n = \frac{u_e(c_e - c_c)}{100s} - c_c E$$

A_n; Net Photosynthesis ($\mu mol \cdot CO_2 \cdot m^{-2}s^{-1}$), u_e; mole flow rate of air entering the leaf chamber ($\mu mol \cdot s^{-1}$), c_e; mole fraction of CO_2 in the leaf chamber ($\mu mol \cdot CO_2 \cdot mol^{-1}$ air), c_c; mole fraction of CO_2 entering in the leaf chamber ($\mu mol \cdot CO_2 \cdot mol^{-1}$ air), s; leaf area (cm^2), E; transpiration ($mmol \cdot H_2O\ m^{-2}s^{-1}$)

This study also performed a regression analysis and obtained photosynthesis curves using SigmaPlot (SPSS Inc.) to estimate precise light-photosynthesis curves and light compensation points.

$$y = ax/(b+x)$$

A light compensation point was obtained when y=0 in the equation above.

Results and Discussion

1. Changes of chlorophyll content

Chlorophyll is the molecule that intervene on photosynthesis; the final substance metabolic process of a plant. Among many kinds of organic environment factors, moisture and sunlights are the vital factors that directly and indirectly affect photosynthesis (Choi, 2001; Han and Sim, 1989).

The analysis result on chlorophyll a, b, total chlorophyll content and chlorophyll a/b according to moisture process in tree species and time is as shown in Table 3.

As a result of chlorophyll characteristic analysis against the tree that has grown from A, B, C and D treatments of different soil moisture environment on *F. rhynchophylla*, the chlorophyll a derived from the month of June showed content of 1.42 mg/g from 0.96 mg/g, while the chlorophyll b showed content of 0.37 mg/g from 0.24 mg/g. While the chlorophyll a showed 1.59 mg/g from 1.22 mg/g in August, the chlorophyll b showed 0.42 mg/g from 0.32 mg/g indicating increment of chlorophyll a and b contents and overall chlorophyll content comparing the month of June to August. This demonstrates the vigorous growth of plants and increase in the content of September's chlorophyll compared to that of August. Consequently, the change of chlorophyll contents of *F. rhynchophylla* has generally increased from June to September. As the soil moisture content decreases, the overall chlorophyll content has decreased including content of both chlorophyll a and b.

As similar tendency to *F. rhynchophylla*, the chlorophyll a content of *F. mandshurica* grown from different treatments of A, B, C, and D also demonstrated increment from 0.89 to 1.67 mg/g and the chlorophyll b showed increment from 0.18 to 0.44 mg/g in the order of June<August<September period. Both *F. rhynchophylla* and *F. mandshurica* showed increment in amount of chlorophyll a and b with the lapse of growing period and the increment amount was higher between August and September than that of between June and August.

The content of chlorophyll usually decreases as the leaves reach the ageing stage from the ripening period and as an adverse environment develops in terms of moisture and nutrient, the decrease of content would be likely to become distinguished (Kwon and Lee, 1994). In this study, the control treatment with plenty of water had shown the higher content of chlorophyll. Several reasons were determined for this outcome in which first, the moisture stress in the soil affected nutrient supply to the young nursery stock second, normal synthesis and protection on chlorophyll was not carried out and third, there was no new chlorophyll creation that would substitute the destroyed chlorophyll.

Unlike the tree species that depend their growth on fixed growth method, *F. rhynchophylla* and *F. mandshurica* are the free growth species that increase chlorophyll content until autumn cause of constant growth of the stem. Also compared to the increase of chlorophyll a the content of chlorophyll b constantly increased at a greater rate (Lee, 2006).

2. Photosynthesis characteristics

In order to investigate photosynthesis characteristics in

Table 3. Seasonal changes of chlorophyll contents in the seedlings of *F. rhynchophylla* and *F. mandshurica* subjected to artificial water treatments.

Season	Treatment	Chlorophyll contents (mg/g f.w.)			Chlorophyll a/b ratio
		a	b	Total	
			Fraxinus rhynchophylla		
June	A	1.42a[Z]	0.37a	1.79a	3.98a
	B	1.21ab	0.30a	1.51ab	4.11a
	C	1.16ab	0.27a	1.43ab	4.79a
	D	0.96b	0.24a	1.20b	3.94a
Aug.	A	1.59a	0.42a	2.00a	3.86a
	B	1.57ab	0.41a	1.98a	4.07a
	C	1.24ab	0.35a	1.59a	3.61a
	D	1.22b	0.32a	1.54a	3.91a
Sep.	A	1.81a	0.58a	2.39a	3.11a
	B	1.61ab	0.51a	2.12ab	3.18a
	C	1.52ab	0.46ab	1.98ab	3.34a
	D	1.24b	0.43b	1.67b	2.92a
			Fraxinus mandshurica		
June	A	1.14a	0.29a	1.43a	5.33a
	B	1.11a	0.29a	1.40a	3.87a
	C	0.98a	0.27a	1.25a	3.81a
	D	0.89a	0.18a	1.07a	5.59a
Aug.	A	1.36a	0.38a	1.74a	3.72a
	B	1.14ab	0.36ab	1.50ab	3.25a
	C	1.09b	0.34b	1.4b	3.37a
	D	0.94b	0.21b	1.15b	4.96a
Sep.	A	1.67a	0.44a	2.11a	3.79a
	B	1.46ab	0.40ab	1.86ab	3.63a
	C	1.46b	0.37ab	1.83b	4.33a
	D	1.52b	0.29b	1.81b	5.12a

Relative soil water: A, 90~78%; B, 70~58%; C, 50~38%; D, 30~18%.
[Z]Means with the same letter within a column are not significantly different at 5% level by Duncan's multiple range test.

accordance with moisture process of *F. rhynchophylla* and *F. mandshurica*, the study adjusted the moisture condition of soil to A, B, C, and D treatments. It also analyzed and compared the photosynthesis characteristics by the species, time and moisture process against grown nursery stock (Table 4). While the variation for light compensation point of *F. rhynchophylla* was not significant to 19.0±0.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in June, 20.0±0.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$, in August and 18.0±1.3$\mu mol \cdot m^{-2} \cdot s^{-1}$ in September in A treatment with fair soil moisture condition, the light compensation point displayed higher in the C and D treatments that have relatively low moisture condition to 49~60 $\mu mol \cdot m^{-2} \cdot s^{-1}$. However, an opposite tendency to the light compensation point was shown in light saturation point.

Variation of the light compensation point of *F. mandshurica* showed decreasing tendency as time lapse similar to *F. rhynchophylla* in which reads 33.0±1.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in June, 27.0±1.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in August and 12.0±1.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in September however, the treatments with relatively lower soil moisture condition showed higher light compensation point. Variation of

Table 4. Effect of artificial water treatment on the photosynthesis characteristic of two deciduous tree species.

Season	Treatment	Light compensation point ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Light saturation point ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Photosynthetic capacity ($\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$)
			Fraxinus rhynchophylla	
June	A	19.0	617.2	4.7
Aug.	A	20.0d[z]	671.9a	4.9a
	B	39.0c	609.4b	2.2b
	C	54.0b	546.9c	1.9c
	D	60.0a	367.2d	0.8d
Sep.	A	18.0d	867.2a	6.6a
	B	35.0c	687.5b	2.4b
	C	49.0b	562.5c	1.4c
	D	58.0a	484.4d	0.5d
			Fraxinus mandshurica	
June	A	33.0	757.8	4.5
Aug.	A	27.0d	914.1a	5.0a
	B	42.0c	710.9b	2.3b
	C	50.0b	664.1c	2.0c
	D	95.0a	578.1d	1.0d
Sep.	A	12.0c	789.1a	6.5a
	B	21.0b	609.4b	2.3b
	C	25.0b	421.9c	1.3c
	D	85.0a	390.6d	0.4d

Relative soil water: A, 90~78%; B, 70~58%; C, 50~38%; D, 30~18%.
[z]Means with the same letter within a column are not significantly different at 5% level by Duncan's multiple range test.

light saturation point also showed similar tendency to that of *F. rhynchophylla*. Moisture stress deteriorated turgor pressure of guard cell which closed stomata and as a result causing shortage of carbon dioxide ultimately affecting on the photosynthesis characteristics (Lee, 2006; Kramer, 1983). The result showed tendency of agreement in *Populus nigra* (Bahari *et al*, 1983), silver maple (Peterson and Bazzaz, 1984) and citrus trees (Phung and Knipling, 1976) in which demonstrated remarkable speed decline and the photosynthesis characteristics due to the affect of moisture stress on closed stomata.

The photosynthetic rate as per growth state of *F. rhynchophylla* and *F. mandshurica* according to artificial moisture process are shown in Fig. 2. While *F. rhynchophylla* showed high photosynthetic rate even in September which elapsed growth time of A treatment with relatively decent moisture condition soil. The B, C and D treatments showed relatively lower photosynthetic rate due to stress derived from insufficient moisture content in the soil. In September, while the A treatment showed 6.6±0.4 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$, the D treatments showed 0.5±0.2 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$ which is 10 folds more difference of photosynthetic rate.

F. mandshurica also showed similar tendency to *F. rhynchophylla* demonstrating photosynthetic rate according to moisture treatments in the order of A>B>C and>D. The photosynthetic rate of month of September showed significant difference as per control group from 0.4±0.1 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$ in the D treatment to 6.5±1.2 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$ in the A treatment.

However for the content of chlorophyll and photosynthetic rates that respond to moisture process demonstrated decrease tendency as the moisture condition of the soil is low and as the stress lasted longer. The results showed even more irregular patterns in September. The reason for this outcome is supposed that the moisture stress in the soil affected nutrient supply to the young

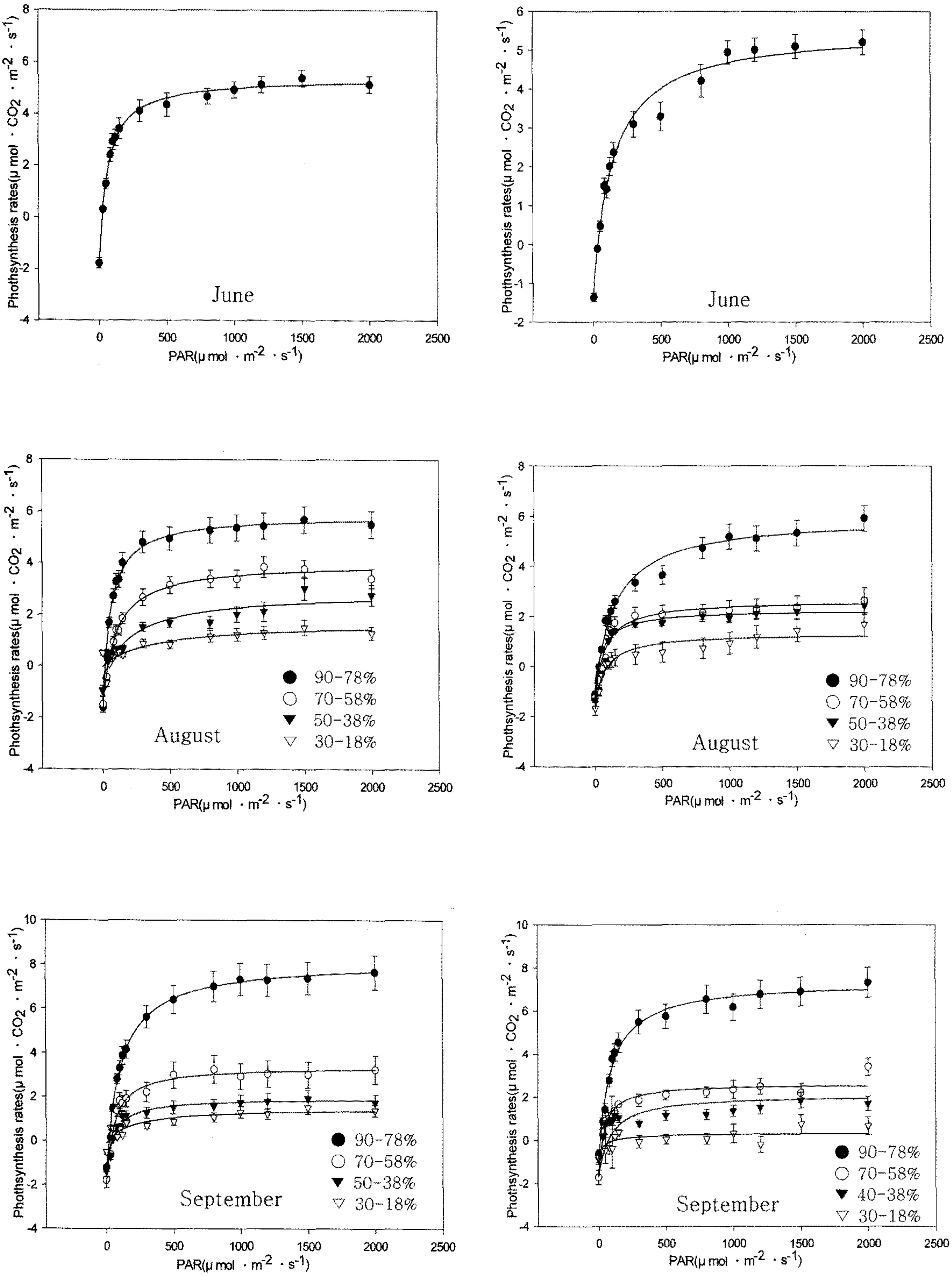

Fig. 2. Effect of artificial water treatment on the photosynthetic rate of two deciduous seedlings (Left, *F. rhynchophylla*; Light, *F. mandshurica*).

nursery stock. And so normal synthesis and protection on chlorophyll was not carried out and there was no new chlorophyll creation that would substitute the destroyed chlorophyll (Kwon and Lee, 1994).

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인위적인 수분처리에 의한 물푸레나무와 들메나무의 엽록소 함량 및 광합성에 미치는 영향

이수원[1] · 최정호[2*] · 정진철[3] · 권기원[4] · 유세걸[1] · 배종향[5]
[1]국립산림과학원 산림생산기술연구소, [2]케너텍(주) 자원개발본부, [3]원광대학교 생명환경학부,
[4]충남대학교 산림자원학전공, [5]원광대학교 원예 · 애완동식물학부

적 요. 인위적인 토양내 수분처리 결과 물푸레나무와 들메나무 모두 6월<8월<9월로 갈수록 엽록소 함량이 증가하였다. 생육기간이 지나는 동안 엽록소 a는 0.89mg/g에서 1.67mg/g로, 엽록소 b는 0.18mg/g에서 0.44mg/g을 나타냈으며, 두 수종 모두 6월보다 8월과 9월에 높은 함량 증가를 보였다. 두 수종의 광합성율을 보면, 물푸레나무는 각 토양수분 처리구마다 광합성율의 차이를 보였으며, 충분한 토양수분처리구(90~78%)의 묘목이 상대적으로 낮은 토양수분조건에서 자란 묘목보다 높은 경향을 나타냈다. 들메나무 역시 물푸레나무와 비슷한 경향을 보였으며, 토양수분이 높을수록 광합성률이 높게 나타났고, 감소할수록 광합성률이 감소하였다. 광합성율의 생육시기별 변화는 두 수종 모두 9월>8월>6월 순으로, 9월에 상대적으로 높은 광합성률을 나타냈다. 이로 미루어보면 두 수종은 생장시기에 상관없이 수분함량이 높은 처리구에서 광합성이 활발히 이루어지는 것으로 생각된다.

주제어 : 광합성률, 광합성 특성, 들메나무, 물푸레나무, 수분처리, 엽록소 함량