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## **Gas Exchange Characteristics of Triploid *Cryptomeria japonica* D. Don**

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Leaf photosynthetic rate, leaf conductance, specific leaf area, stomatal size and stomatal density of triploid and diploid cultivars of *Cryptomeria japonica* D. Don were measured in order to clarify gas exchange characteristics of triploid *C. japonica*. Cultivar cv. Measa (diploid : 2x), cv. Ayasugi (2x) and cv. Hinode (triploid : 3x) were used for the measurements. The photosynthetic rate on a leaf area basis was 50% higher in the triploid than in the diploids. Photosynthetic rate on a leaf weight basis was not significantly different among the cultivars. Specific leaf area (SLA) in the diploids were 30% higher than in the triploid. Mesophyll width in the triploid was 57% higher than in the diploids. In the leaf conductance measurement, 5.2 times more conductance in the triploid than in the diploids was observed. Stomatal density in the triploid was 27% lower than the diploid. The difference in leaf conductance between the triploid and the diploids was mainly caused by difference in stomatal conductance. Both of the stomatal size and mesophyll width in the triploid were 40 to 60% higher than in the diploids. The difference in the photosynthetic rate on a leaf area basis and in the leaf conductance values between the triploid and the diploids were induced by the difference in mesophyll and stomatal cell size in proportion to the ploidy level.

### INTRODUCTION

*Cryptomeria japonica* D. Don is one of most valuable silvicultural species in Japan. Many cultivars of *C. japonica* have been selected by their growth characteristics. However, the evaluation of growth characteristics for selection is time consuming. The present concern is to establish a method for the rapid estimation of the value of a cultivar for selection breeding based on the ecophysiological characteristics of *C. japonica*.

Recently, the potential use of triploid *Cryptomeria japonica* as breeding material has been recognized. It was clarified that cv. Hinode and cv. Urasebaru which are *C. japonica* conventional cultivar were triploid (Matsuda and Miyajima, 1977). A lot of triploid cultivars were included as elite cultivars by the National Project on Elite Tree Selection Breeding (Kondo, 1985). Chromosomal investigations and the external leaf form characteristics of the triploid *C. japonica* cultivars have been reported, but gas exchange characteristics have not been studied.

The effects of polyploidy on gas exchange functions have been researched in many crop plant species. Photosynthetic rates increased with ploidy level in *Panicum virgatum* (Warner *et al.*, 1987), *Festuca arundinacea* (Joseph *et al.*, 1981) and *Agropyron cristatum* (Frank, 1980). However, photosynthetic rates decrease or do not change in higher ploidy levels in *Triticum* (Austin *et al.*, 1982), *Datura stramonium* (Cukrova and Avratovscukova, 1968). In many studies the photosynthetic rates of polyploids were

expressed in terms of leaf area, and were not analyzed in relation to changes in leaf morphology.

The present study was undertaken to investigate the difference in the photosynthetic rate and the stomatal conductance between diploid and triploid cultivars of *C. japonica*, and to estimate the effect of variation in leaf morphology on gas exchange characteristics.

## MATERIALS AND METHODS

### Plant materials

Rooted cuttings of *C. japonica* cv. Hinode as triploid clone, cv. Measa and cv. Ayasugi as diploid clone were used for all measurements in this study. The cuttings of cv. Hinode were collected at Kyushu Regional Breeding Office, Forest Tree Breeding Institute at Kumamoto Prefecture, center Kyushu, Japan, and rooted at the nursery of Kyushu university, northern Kyushu, in 1991. The rooted cuttings of cv. Ayasugi and cv. Measa were grown at the Forest Research and Instruction Station of Kumamoto Prefecture in 1991. All rooted cuttings were transplanted to clay pots at the nursery of Kyushu university in April 1992.

### Gas exchange measurement

Current shoots of the rooted cuttings were cut in distilled water for gas exchange measurements on the 4th October 1992. CO<sub>2</sub> exchange rates and stomatal conductances in each sample were measured on current shoots of the detached shoots using a gas exchange rate analyzer (LCA-3, ADC, UK). Four shoots per cultivar were measured as replicates. Photosynthetic photon flux density during measurements was  $1600 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  at surface of the chamber. Temperature at leaf surface in the chamber was  $26 \pm 0.5^\circ\text{C}$ . Vapor pressure deficit in the chamber during the measurements was  $1.8 \pm 0.2 \text{ KPa}$ . CO<sub>2</sub> concentration of output air was regulated at 350 to 360 ppm using ambient air.

### Leaf morphology

The sample leaves were printed in OHP films after cutting off from the xylem. The areas of images of the leaves on the OHP films were measured using the leaf area meter (Li-cor, USA). Dry weights of the sample leaves were measured after leaf area measurements. Sump method was used to measure stomatal diameter and the stomatal density of leaves of each cultivar. Mesophyll width at the base of the leaves was measured with a micrometer. Five leaves were used to measure stomatal density, stomatal diameter and mesophyll width for a cultivar.

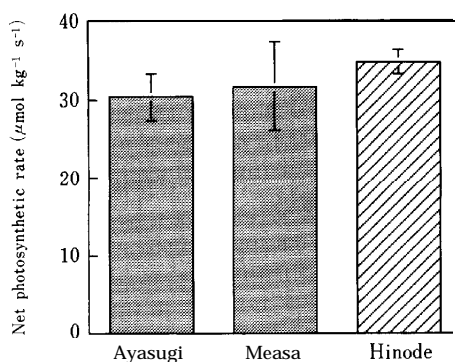
## RESULTS AND DISCUSSION

Figure 1 and Fig. 2 show light saturated net photosynthetic rate ( $P_n$ ) in terms of leaf weight and leaf area in each cultivar. The difference in the  $P_n$  per leaf area and weight between two diploid cultivars was not significant.  $P_n$  based on unit leaf area

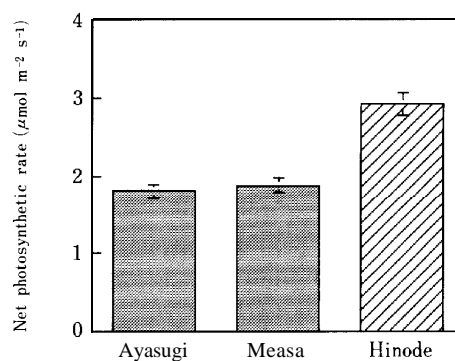
was 58% higher in the triploid than in the diploids (Fig. 2). However, based on per unit leaf weight, there was no significant difference between two ploidy levels (Fig. 1).

Figure 3 shows the leaf conductance values in each cultivar. The leaf conductance in the triploid was 5.6 times more than in the diploids.

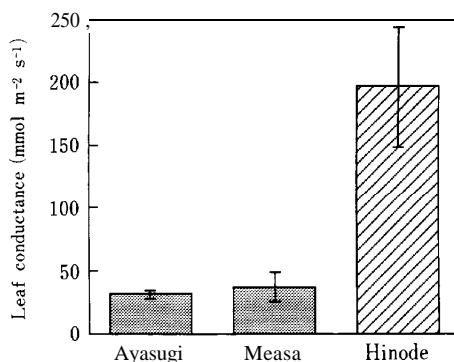
Table 1 shows leaf morphological parameters of each cultivar. The SLA was about 40% higher in the diploids than in the triploids, and mesophyll width was 57% higher in triploid than in the diploids. The lower SLA in the triploid is caused by the higher mesophyll width. Stomatal density in the diploids was 1.4 times as much as the



**Fig. 1.** Net photosynthetic rates per leaf weight of diploid (cv. Measa and cv. Ayasugi) and triploid (cv. Hinode) *C. japonica*. Steady state  $\text{CO}_2$  exchange rates were measured in saturated PPFD. Bar shows standard deviation.



**Fig. 2.** Net photosynthetic rates per leaf area of diploid (cv. Measa and cv. Ayasugi) and triploid (cv. Hinode) *C. japonica*. Steady state  $\text{CO}_2$  exchange rates were measured in saturated PPFD. Bar shows standard deviation.



**Fig. 3.** Leaf conductance of diploid (cv. Measa and cv. Ayasugi) and triploid (cv. Hinode) *C. japonica*. Steady state H<sub>2</sub>O exchange rates were measured at  $26 \pm 0.5^\circ\text{C}$  temperature,  $1.8 \pm 0.2$  KPa vapor pressure deficit. Bar shows standard deviation.

**Table 1.** Morphological Comparison of Diploid and Triploid *C. japonica* cultivars

Parameter	Cultivars			3x/2x
	Measa (2x)	Ayasugi (2x)	Hinode (3x)	
Specific Leaf Area (cm <sup>2</sup> /g)	168.4 $\pm$ 6.1	169.2 $\pm$ 3.8	119.4 $\pm$ 1.8	0.71 <sup>**</sup>
Mesophyll width ( $\mu\text{m}$ )	72 $\pm$ 6	81 $\pm$ 5	120 $\pm$ 9	1.57 <sup>**</sup>
Stomatal density (Num./cm <sup>2</sup> )	4401 $\pm$ 185	4255 $\pm$ 112	3181 $\pm$ 81	0.73 <sup>**</sup>
Guard cell length ( $\mu\text{m}$ )	33 $\pm$ 2	28 $\pm$ 2	44 $\pm$ 2	1.44 <sup>**</sup>

<sup>\*\*</sup> : significant difference between ploidy levels at 0.5% level

triploid. The diameter of Stomatal guard cells of the triploid was about 1.4 times as long as the diploids. The ratio of the triploid to the diploids on the mesophyll width and guard cell length were proportion to the ploidy levels of the cultivars.

The ratio of the photosynthetic rate based on leaf area between triploid and diploids was equivalent to the ratio of mesophyll width. However, The ratio of the leaf conductance was much larger than the ratio of stomatal guard cell diameter. Although cell size ratio between triploid and diploid follows the ploidy level, leaf conductance ratio is exponential to the 4th power.

Warner et al. (1987) reported that photosynthetic rate per leaf area of *Panicum virgatum* increased by 40%, and the amount of DNA and enzyme activity doubled with a doubling of chromosome number, but the size of cells increased only 15 to 20%. Moreover, Johnson et al. (1987) reported that the photosynthetic rates in diploid and tetraploid Triticum were larger than hexaploid. Therefore, it is generally necessary to consider factors like enzyme activity per cell volume, to discuss the relationship between the chromosomal numeric variations and the gas exchange characteristics.

The results in this paper show that the proportionality of cell size to ploidy level is closely related to the gas exchange characteristics of a cultivar.

It must be noted, however, that Saito et **al.** (1958) reported poor growth in triploid *C. japonica*. Thus, although the findings of this study indicate a higher photosynthetic rate in the triploid cultivar used, further study is needed to clarify if polyploidy in *C. japonica* confers advantage.

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