

Full Length Research Paper

# Variation in photoinhibition and photoprotection between seedlings and saplings leaves of *Taxus cuspidata* under high irradiance

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The differences in chloroplast pigments, gas exchange and photosystemII (PSII) photochemistry as well as xanthophyll in seeding and sapling leaves of *Taxus cuspidata* grown in full sunlight were examined. Compared with the sapling leaves, the chlorophyll content, photosynthetic capacity and light intensity for saturation of photosynthesis were lower in seeding leaves. The response curves of PSII photochemistry demonstrated that both seeding and sapling leaves occurred a down-regulation of PSII photochemistry at high irradiance, more serious down-regulation being examined in seeding leaves. And the down-regulation of PSII photochemistry occurred significantly when measured at midday, indicating that photoinhibition occurred heavily in seeding leaves when exposed to high light. The actual PSII efficiency ( $\Phi_{PSII}$ ) and the efficiency of excitation capture by open PSII centers drastically decreased with the increase of non-photochemical quenching (NPQ) at midday. The photorespiration rate in seeding leaves was lower than that in sapling leaves under high irradiance. The results indicated that the xanthophyll cycle was activated in both the seeding and sapling leaves at midday and an increase of de-epoxidation were observed, but a little higher level of de-epoxidation was measured in seeding leaves. The xanthophyll cycle may play an important role in the dissipation of excess light energy associated with NPQ to avoid photodamage. Our results suggested that photoinhibition occurred in seeding leaves significantly due to lower capacity of CO<sub>2</sub> assimilation, photorespiration and the light intensity for saturation of photosynthesis, as well as the lower PSII photochemistry at high irradiance; therefore the *T. cuspidata* seeding could not adapt to growing at high irradiance.

**Key words:** Japanese yew (*Taxus cuspidata* Sieb, et Zucc.), photosynthesis, chlorophyll fluorescence, photorespiration, xanthophyll cycle.

## INTRODUCTION

Japanese yew (*Taxus cuspidata* Sieb, et Zucc.) is a rare relic plant of the 'tertiary period', which has a wide geographical distribution (Potenko, 2001). It grows in vegetated mixed forests in mountainous regions. Taxol, an effective anticancer drug extracted from the bark of Japanese yew, receives attention (Kobayashi et al., 1994). Japanese yew is a declining species, but conservation strategies have been developed (Potenko, 2001). As we known, high light may decrease the rate of

photosynthesis in plant which may cause the photoinhibition (Müller et al., 2001; Huang et al., 2006). Japanese yew is a shade-tolerant species (Iszkulo and Boratynski, 2006). The saplings can survive in both shady and sunny environments, but the seedlings are always observed under the canopy of mature trees (Iszkulo and Boratynski, 2006). Therefore, when the Japanese yew seedlings are exposed to the high light, photoinhibition could occur in Japanese yew seeding leaves. However,

plants have developed some photoprotective mechanisms to protect the photosynthesis apparatus against photodamage (Lu et al., 2003; Chow, 1994; Anderson et al., 1997). Dissipation of excess excitation

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energy as heat in order to minimize photodamage to PSII reaction centers is well known to be one of the mechanisms for the protection of the photosynthetic apparatus, which involves the xanthophyll cycle (Guo et al., 2009).

In the xanthophyll cycle, excess light energy absorbed by antennae complexes of photosystem II is converted to heat, which prevent the formation of reactive oxygen. In this process, violaxanthin (V) is converted to zeaxanthin (Z) and antheraxanthin (A) under conditions of excess excitation energy (Demmig-Adams and Adams, 1992; Gilmore, 1997; Horton et al., 1996). And photorespiration pathway is reported as a very important photoprotection mechanism against photooxidation and photoinhibition (Kozaki and Takeba, 1996; Jiang et al., 2006; Niyogi, 1999). Photorespiration could act as a sink for excess excitation energy in photosynthetic apparatus when CO<sub>2</sub> assimilation is reduced (Niyogi, 1999). In this study, we conducted an experiment to determine the differences between *T. cuspidata* seeding and sapling leaves in the CO<sub>2</sub> assimilation capacity, photorespiration capacity and xanthophyll cycle-dependent energy dissipation under high irradiance and whether the *T. cuspidata* seeding could adapt to full sun light.

## MATERIALS AND METHODS

### Plant material

The research was carried out from March to August, 2010 in the Botanical Garden of North East Forestry University. The 30 *T. cuspidata* seedlings of 4 years and the 16 ones of 15 years which grew in plastic pots (25 cm in diameter and 20 cm in height; 80 cm in diameter and 70 cm in height, respectively) were transplanted from 70% PPFD (photosynthetic photon flux density) of full sunlight to 90% PPFD of full sunlight. After 4 weeks under 90% PPFD of full sunlight, they were moved to the full sunlight. Six weeks later when the *T. cuspidata* seedlings and saplings were acclimated to full sun light, the current-year leaves from the mid-crown on the south side of each tree were studied as the experimental materials.

### Gas change measurements

Photosynthetic rate-photosynthetic photon flux density (Pn-PPFD) response curves were made at leaf chamber temperature of 30° and at 350  $\mu\text{molmol}^{-1}\text{CO}_2$  with an open gas exchange system (Li-6400). PPFDs were fixed in a sequence of 1800, 1600, 1200, 800, 600, 400, 200, 100, 500  $\mu\text{molmolm}^{-2}\text{s}^{-1}$ . Photosynthetic rate was monitored at two O<sub>2</sub> concentrations: 21% O<sub>2</sub> + 350  $\mu\text{molmol}^{-1}\text{CO}_2$  and 2% O<sub>2</sub> + 350  $\mu\text{molmol}^{-1}\text{CO}_2$  under 1400  $\mu\text{molmolm}^{-2}\text{s}^{-1}$  PPFD and this was used to calculate photorespiration.

### Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured with a pulse-modulated fluorometer (FMS-2, Hansatech, UK). Before each measurement, the sample leaf was dark-adapted for 35 min with dark leaf clips. To determine the F<sub>o</sub> (initial fluorescence), the low modulated measuring light ( $<0.1 \text{ m}^{-2}\text{s}^{-1}$ ) was turned on and F<sub>o</sub> was recorded. Then the sample leaf was exposed to a 0.7 s saturating white light

( $>3000 \text{ m}^{-2}\text{s}^{-1}$ ) to obtain the F<sub>m</sub> (maximal chl fluorescence). F<sub>v</sub>/F<sub>m</sub> (the maximum quantum yield of photosystemII; F<sub>v</sub>, the variable Chl fluorescence yield is defined as F<sub>m</sub> - F<sub>o</sub>) was calculated automatically. F<sub>s</sub> (the steady-state fluorescence) and F<sub>m</sub>' (the maximum Chl fluorescence level) during exposure to illumination were also measured. The actual PSII efficiency ( $\Phi\text{PSII}$ ) was calculated as (F<sub>m</sub>'-F<sub>s</sub>)/F<sub>m</sub>' (Genty et al., 1989). Non-photochemical quenching (NPQ) was calculated as (F<sub>m</sub>/F<sub>m</sub>')<sup>-1</sup> according to Bilger and Björkman (1990).

To examine the light response curves for the fluorescence parameters of *T. cuspidata* sapling leaves and seeding leaves, the F<sub>o</sub> was measured at first and then a saturating pulse was applied to determine the F<sub>v</sub>/F<sub>m</sub>. The actinic light was increased in a sequence of 100, 200, 400, 600, 800, 1000 and 1400 in steps. Each PFD was maintained at least 10 min.

### Pigment determination

The content of chlorophyll in leaf were extracted with 80% acetone, being analyzed with a UV-2800 system (Hitachi, Japan) according to Lichtenthaler (1987). Leaf samples were taken at morning. The content of carotenoid components of xanthophyll was extracted with 100% acetone under the ice-cold condition. Then the extracts were filtered through a 0.45  $\mu\text{m}$  filter. Leaf samples were taken at predawn and midday. Afterwards, they were immediately frozen into liquid nitrogen. The content of the carotenoid components of xanthophyll were analyzed in the method described by Thayer and Björkman (1990) for 5 times.

### Statistical analyses

Data of measurements were analyzed by using SPSS 10.0. The least significant differences between the means were calculated at 95% confidence level. Plots and fit curves were performed by using Sigmaplot10.0. Unless otherwise indicated, the significant differences between seedlings and saplings were given at P<0.05.

## RESULTS

### Differences in chlorophyll content

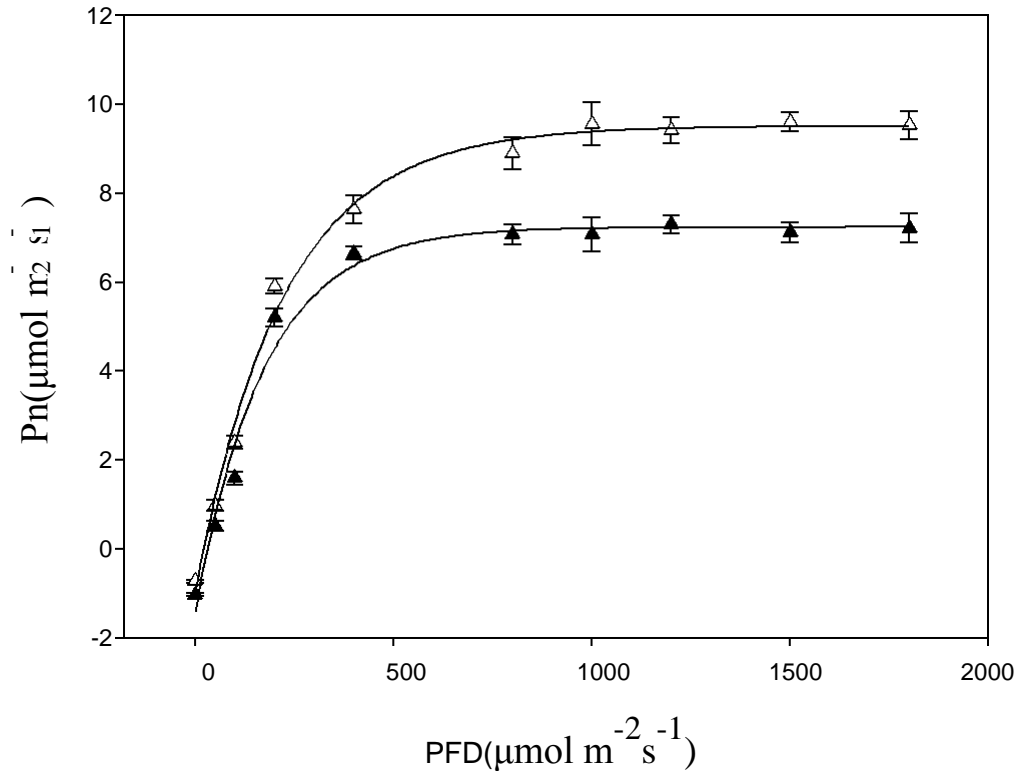
The Chl *a*, Chl *b* and total *a* + *b* content in saplings leaves were significantly higher than those in seedlings leaves. The ratios Chl *a*/Chl *b* did not show significant differences between saplings leaves and seedlings leaves. The result demonstrated that the content of Chl *a* was higher than that of Chl *b* under the high light (Table 1).

### Differences in photosynthesis and photorespiration

There were significant differences between *T. cuspidata* seeding and sapling in CO<sub>2</sub> assimilation capacity and photorespiration. Measurements of light response curves for photosynthesis of *T. cuspidata* seeding and sapling leaves show that the maximum photosynthetic rates were  $7.22 \pm 0.33$  and  $9.6 \pm 0.21 \mu\text{molm}^{-2}\text{s}^{-1}$  in seedlings and saplings leaves, respectively (Figure 1). Sapling leaves exhibited higher saturation light of photosynthetic rate

**Table 1.** Differences in chlorophyll pigments between *T. cuspidata* seedling and sapling leaves. Each value is means of  $\pm$  S.E. n = 5.

Variable	Chl a ( $\text{mg g}^{-2}$ FW)	Chl b ( $\text{mg g}^{-2}$ FW)	Chl a+b ( $\text{mg g}^{-2}$ FW)	Chl a/b ( $\text{mg m}^{-2}$ )
Seedling	$314 \pm 10^a$	$88 \pm 2^a$	$443 \pm 7^a$	$3.56 \pm 0.06^a$
Sapling	$362 \pm 7^b$	$98 \pm 1^b$	$487 \pm 4^b$	$3.62 \pm 0.09^a$



**Figure 1.** Light response curves for photosynthesis of *T. cuspidata* seedlings and saplings leaves measured at 26°C and the 350  $\mu\text{mol mol}^{-1}$  CO<sub>2</sub> in the chamber. ( $\Delta$ ) and ( $\blacktriangle$ ) represent sapling leaves and seedling leaves, respectively. Values are means  $\pm$  S.E., n = 3 – 5.

than that of seedling leaves. Sapling leaves had higher CO<sub>2</sub> assimilation capacity under high irradiance. Similarly, photorespiratory in sapling leaves also showed higher than that in seedling leaves (Figure 2).

#### Response of Chl fluorescence parameters to changes in irradiance

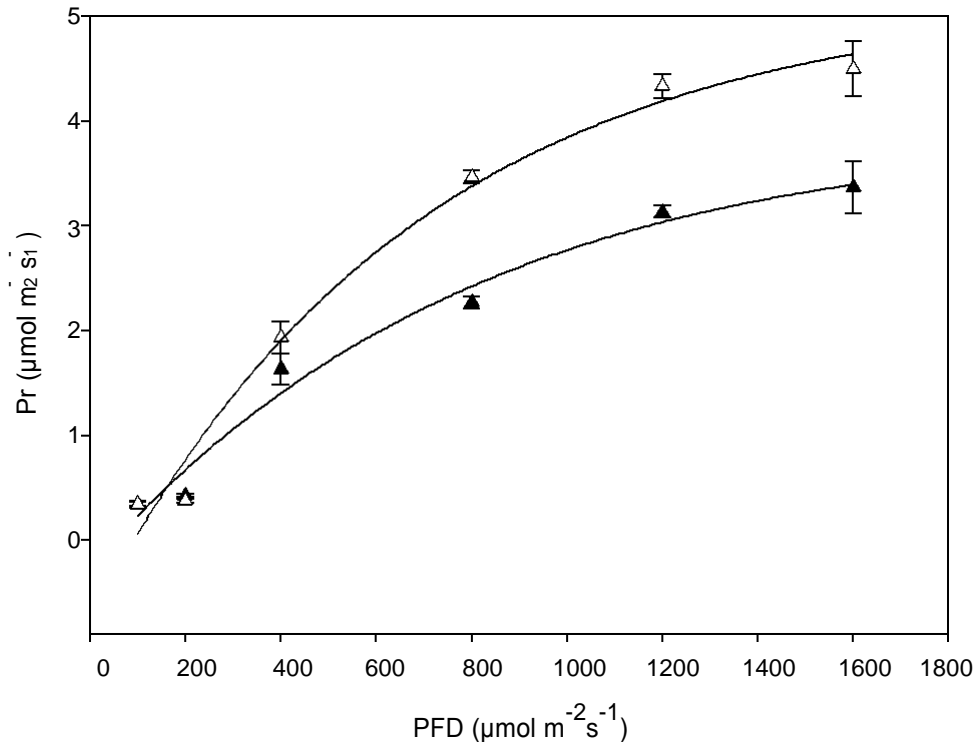
With the irradiance increasing, the decrease in  $\Phi\text{PSII}$  and  $F_v/F_m'$  and an increase in NPQ were observed in seedling and sapling leaves, but the sapling leaves had higher  $\Phi\text{PSII}$  and  $F_v/F_m'$  than the seedling ones. However, NPQ in seedling leaves was significantly higher than sapling ones. The results showed that a greater down-regulation of PSII efficiency in seedling leaves in high light (Figure 3).

#### Fluorescence parameters at predawn and midday

A significant decline in  $F_v/F_m$ ,  $\Phi\text{PSII}$  and  $F_v/F_m'$  were observed at midday in the seedling and sapling leaves, but a considerable increase in NPQ. Compared with seedling leaves, sapling leaves showed higher values for  $F_v/F_m$ ,  $\Phi\text{PSII}$  and  $F_v/F_m'$  and lower values for NPQ at midday (Table 2).

#### The xanthophyll cycle under high irradiance

There were significant differences between Japanese yew in the content of xanthophyll. We observed that the relative xanthophyll pool size  $(A + V + Z)/\text{Chl}$  in seedling leaves was higher than that in sapling leaves (Figure 4A, B and C). Compared with sapling leaves, the de-



**Figure 2.** Light response curves for photorespiratory of *T. cuspidata* seedlings and saplings leaves measured at 26°C. ( $\Delta$ ) and ( $\blacktriangle$ ) represent sapling leaves and seedling leaves, respectively. Values are means  $\pm$  S.E.,  $n = 3 - 5$ .

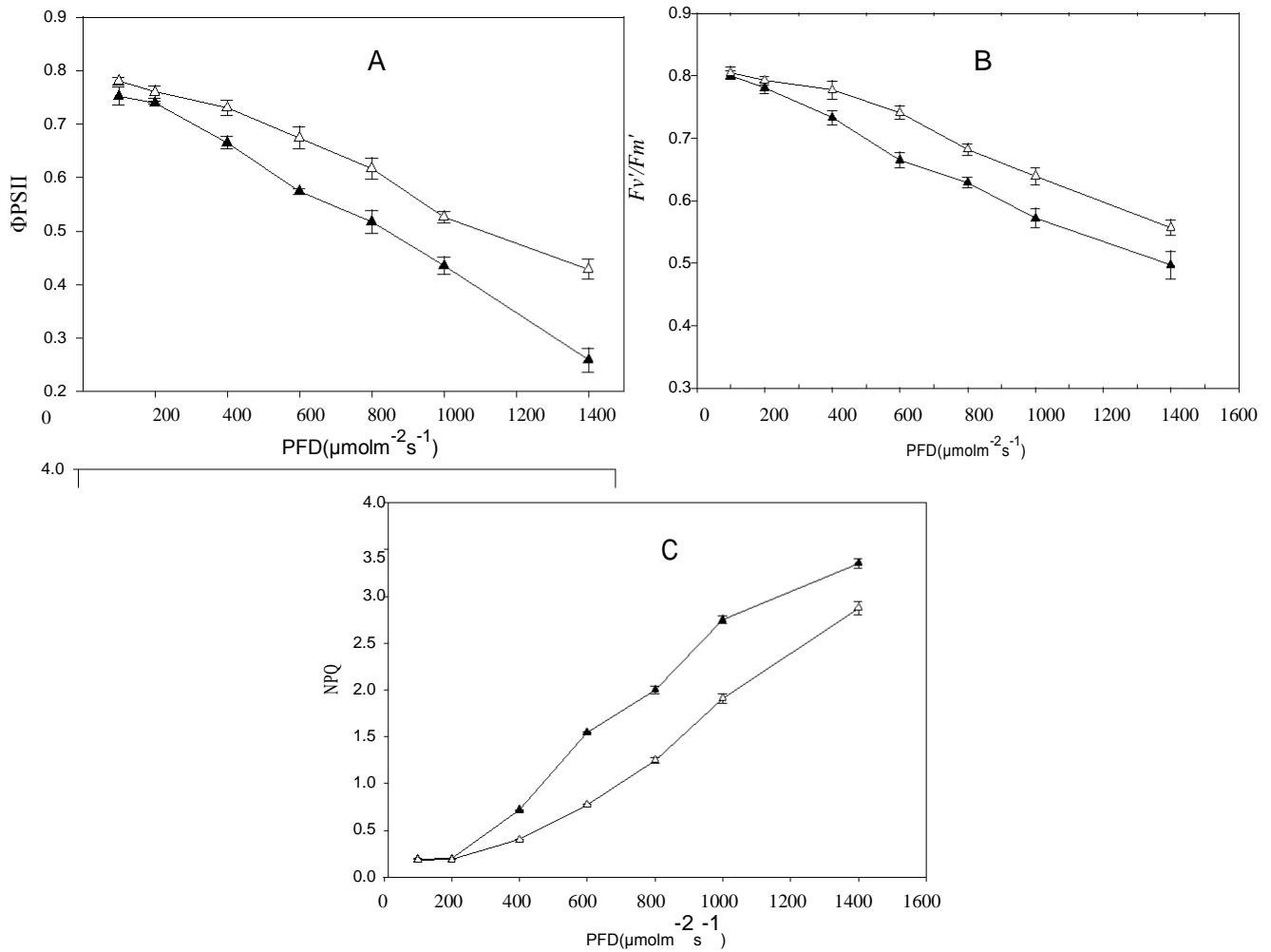
epoxidation components of the xanthophyll cycle pigments were more increased in seedling leaves at midday. And the results showed an increase in  $(A + Z)/(A + Z + V)$  ratio in seedling and sapling leaves at midday.

## DISCUSSION

The data that sapling leaves had higher chlorophyll content, Chl*a*/Chl*b* ratio (Table 1) and photosynthetic capacity (Figure 1) indicated that sapling leaves had a more developed photosynthetic apparatus, which more excited energy would be used in  $\text{CO}_2$  assimilation rather than dissipated. The long exposure to high irradiance levels is a major source of stress to the photosynthetic apparatus (Genty et al., 1989). When  $\text{CO}_2$  assimilation is restricted, photorespiration also acts as a key role in the protection of leaves against high irradiation and uses energy. Sapling leaves had more capacity to allocate excited energy to photorespiration than seedling ones at high irradiance (Figure 2). Increased allocation of excited energy of photorespiration can maintain the utilization of excited energy by allowing metabolism to continue using the products of photosynthetic electron transport. This can mitigate the deleterious effects such as photodamage. The maximal efficiency of PSII photochemistry ( $F_v/F_m$ ) showed only a slight decrease in

seedling leaves when measured at predawn, indicating that seedling leaves had almost the same primary photochemistry as sapling leaves (Table 2), so the activity of PSII may not be the limiting step of photosynthesis in seedling leaves.

With an increasing series of irradiances, the values of  $\Phi_{\text{PSII}}$  and  $F_v/F_m$  decreased gradually (Figure 3A, B and C). However, decrease in PSII efficiency ( $\Phi_{\text{PSII}}$ ) and the efficiency of excitation energy captured by open PSII centers ( $F_v/F_m$ ) in seedling leaves revealed a down-regulation of PSII in the light-response curves. The changes in the light response curves of PSII photochemistry in seedling leaves also showed higher stepwise increases in NPQ at high PFDs. This demonstrated that seedling leaves had to dissipate excess excitation energy as more heat when exposed to high light. It has been reported that xanthophyll cycle is an important photoprotection mechanism correlated to energy dissipation in plants to avoid photodamage. The data demonstrated that a 'little more' de-epoxidation components ( $A + Z$ ) were observed in seedling leaves than that in sapling leaves when measured at midday, which was associated with NPQ. The results showed that an increase in NPQ in both seedling and sapling leaves at midday was associated with an increase in content of ( $A + Z$ ) and increase in  $(A + Z)/(V + A + Z)$  ration. The higher content of ( $A + Z$ ) and the higher  $(A + Z)/(V + A + Z)$  ration



**Figure 3.** Responses of actual PSII efficiency ( $\Phi_{PSII}$ , A), the efficiency of excitation capture by open PSII reaction centers ( $Fv'/Fm'$ , B) and non-photochemical quenching (NPQ, C) to PFDs in *T. cuspidata* seedling ( $\blacktriangle$ ) and sapling ( $\triangle$ ) leaves. Values are means  $\pm$  S.E.,  $n = 3$ .

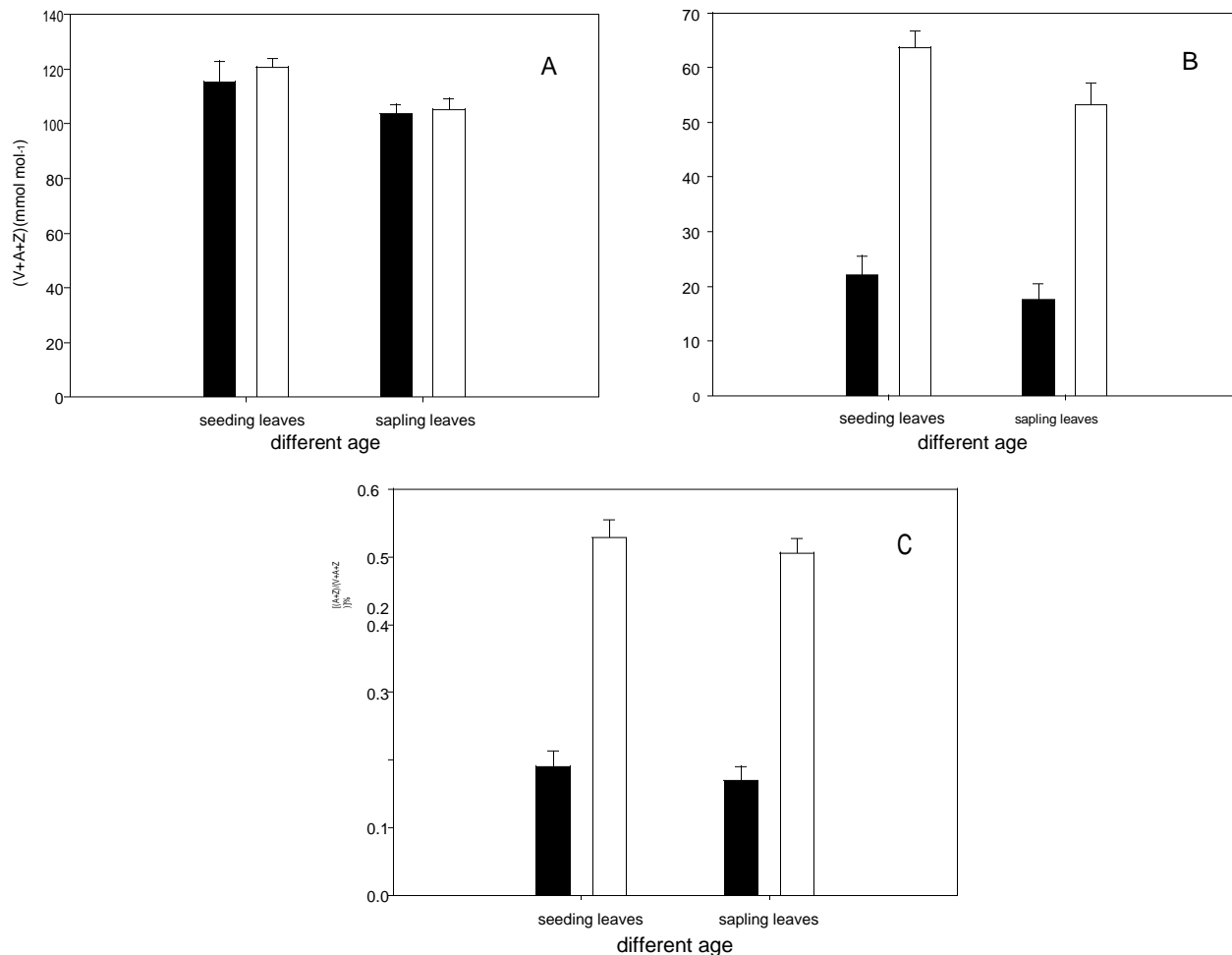
**Table 2.** Differential changes of chlorophyll fluorescence ratios in the maximal efficiency of PSII photochemistry ( $Fv/Fm$ ), actual PSII efficiency ( $\Phi_{PSII}$ ), the efficiency of excitation energy capture by open PSII centers ( $Fv'/Fm'$ ) and non-photochemical quenching (NPQ) in *T. cuspidata* seedling and sapling leaves at predawn and midday with PFD  $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Values are means  $\pm$  S.E.,  $n = 4$ .

Variables	Seedling		Sapling	
	Predawn	Midday	Predawn	Midday
$Fv/Fm$	$0.8 \pm 0.01$	$0.655 \pm 0.02$	$0.841 \pm 0.04$	$0.798 \pm 0.01$
$\Phi_{PSII}$	$0.587 \pm 0.01$	$0.344 \pm 0.02$	$0.616 \pm 0.01$	$0.476 \pm 0.02$
$Fv'/Fm'$	$0.695 \pm 0.01$	$0.483 \pm 0.02$	$0.816 \pm 0.154$	$0.71 \pm 0.01$
NPQ	$1.37 \pm 0.04$	$2.702 \pm 0.133$	$1.147 \pm 0.02$	$2.227 \pm 0.07$

at high irradiance might act as a strengthened acclimation to cope with excess irradiance.

In conclusion, seedling leaves can dissipate the excess energy by xanthophyll cycle, but photoinhibition occurred

in seedling leaves due to lower capacity of  $\text{CO}_2$  assimilation and photorespiration and the light intensity for saturation of photosynthesis as well as the lower PSII photochemistry at high irradiance.



**Figure 4.** Changes of xanthophyll cycle pigment pool size, the de-epoxidation components per Chl, and conversion state of the xanthophyll cycle  $(A + Z)/(A + Z + V)$  in *T. cuspidata* seedling and sapling leaves. Samples were taken at predawn (■black bars) and at midday (□empty bars). Values are means  $\pm$  S.E.,  $n = 3$ .

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