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Effects of osmotic- and high-light stresses on PSII efficiency of attached and detached leaves of three tree species adapted to different water regimes

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Abstract

Abscisic acid (ABA), an important chemical signal from roots, causes physiological changes in leaves, including stomata closure and photoprotection. Furthermore, endogenous ABA concentration in leaves and stomatal behavior vary with the species adapted to different water regimes. In this study, *Ficus microcarpa*, a hemiepiphyte, *Salix warburgii*, a hygrophyte, and *Acacia confusa*, a mesophyte, were used to elucidate the effects of leaf detachment on photosystem II (PSII) efficiency under osmotic- and high-light stresses. Results indicate that, under osmotic- and high-light stresses, PSII efficiency of the detached leaves was lower than that of the attached leaves for all three tree species, when compared at the same levels of stomatal resistance and leaf water potential. Exogenous ABA could mitigate the PSII efficiency decrease of detached *F. microcarpa* leaves under osmotic- and high-light stresses. Yet, the osmotic stress could raise endogenous ABA concentration in the attached, but not in the detached *F. microcarpa* leaves. In addition, partial root-zone drying exerted a significant effect on stomatal behavior but not on the water status of *F. microcarpa* leaves. These observations imply that the stronger ability of PSII in the attached leaves of *F. microcarpa* under osmotic- and high-light stresses was probably due to the protective action of ABA from roots. On the contrary, endogenous ABA level of *S. warburgii* leaves was very low. In addition, partial root-zone drying produced no significant effect on its stomatal behavior. Therefore, PSII in attached *S. warburgii* leaves was possibly protected from the damaging effects of excess absorbed energy by signals other than ABA, which were transported from the roots.

Additional key words: abscisic acid; *Acacia confusa*; chlorophyll fluorescence; *Ficus microcarpa*; osmotic stress; *Salix warburgii*.

Introduction

At the whole-plant level, the effect of stress is usually perceived as a decrease in photosynthesis and growth (Cornic and Massacci 1996). Osmotic stress, one of the most important limiting factors for photosynthesis, can result from water deficit, salinity, and low temperature (Weng 2000, Wang *et al*. 2003). Under osmotic stress, plants often close their stomata to reduce water consumption, with subsequent restriction of CO_2 diffusion into leaves and a decrease of the dark reaction of Calvin cycle (Stuhlfauth *et al*. 1990, Martin and Ruiz-Torres 1992, Lawlor and Cornic 2002). Moreover, reduced water potential of plant tissues also affects mesophyll metabolism by decreasing the efficiency of light energy conversion and/or activity of enzymes involved in CO_2 fixation (Stuhlfauth *et al*. 1990, Martin and Ruiz-Torres 1992, Lawlor and Cornic 2002). In some cases, stomatal closure and depression of Calvin cycle often occur prior to the inhibition of the photosystems, particularly PSII (Stuhlfauth *et al*. 1990, Martin and Ruiz-Torres 1992), leading to the absorption of more photons than they can consume (Stuhlfauth *et al*. 1990, Valladares and Pearcy 1997). This excess absorbed energy could cause photoinhibition by generating reactive oxygen species (ROS) that damage many cellular components, including the photosystems (Powles 1984, Hideg *et al*. 1998). Plants have evolved mechanisms to protect the photosynthetic apparatus against photoinhibition, such as enhancing the xanthophylls cycle to dissipate the excess energy, and promoting the efficiency of antioxidant system to diminish the deleterious effects of ROS (Demmig-Adams and Adams 1996, Niyogi 1999, Logan *et al*. 2006).

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Abbreviations: ABA − abscisic acid; Chl ‑ chlorophyll; F_v/F_m − potential quantum efficiency of PSII; PPFD − photosynthetic photon flux density; PSII − photosystem II; $\Delta F/F_m'$ − actual quantum efficiency of PSII; Ψ_w – leaf water potential.

Detached leaves, especially of trees, have been convenient materials for many plant physiological, phytopathological, and entomological studies (Potvin 1985, Percival and Fraser 2001, Weng *et al*. 2009). However, it has been well known that some signals from roots, *e.g.* chemical, hydraulic, and electrical signals, may lead to physiological changes in leaves (Mancuso and Mugnai 2006, Jia and Zhang 2008). Reports also demonstrated that, even with only a part of roots exposed to drying soil and nonhydraulic limitation in shoots, stomatal conductance, and leaf growth could be regulated by signals from drying roots (Davies and Zhang 1991, Dodd 2005, Jia and Zhang 2008). Among root-to-shoot signals, ABA, a plant hormone, plays a main role in inducing stomatal closure and leaf senescence when roots are exposed to water deficit and osmotic stress (Dodd 2005, Mancuso and Mugnai 2006, Jia and Zhang 2008, Dodd *et al*. 2009). It has also been reported that ABA may protect the photosynthetic apparatus against photoinhibition by enhancing the xanthophyll cycle (Beckett *et al*. 2000, Sharma *et al*. 2002, Jia and Lu 2003) and inducing an antioxidative defence (Jiang and Zhang 2001, Agarwal *et al*. 2005, Lu *et al*. 2009). In addition, ABA also affects the expression of many photosynthetic and high-light-responsive genes (Giraudat *et al*. 1994, Bray 2002, Bechtold *et al*. 2008).

Thus, detached leaves, with their transport severed and lacking certain signals from roots, may exhibit physiological responses different from attached leaves, when exposed to osmotic- and high-light stresses (Nobel and De la Barrera 2002). However, few studies have been carried out by monitoring over a period of time the performance of the attached and detached leaves to elucidate the effect of leaf detachment on PSII efficiency (Potvin 1985, Percival and Fraser 2001). Among these studies, Potvin (1985) reported that, under chilling, chlorophyll (Chl) fluorescence values of the detached leaves from 4 species were lower than those of the attached ones. On the contrary, Percival and Fraser (2001) did not detect any detrimental effects on Chl fluorescence values when the leaves were assessed 72 h following freezing and salinity treatments. Thus, the effects of leaf detachment on PSII efficiency under osmotic- and high-light stresses are still unclear and worth of investigation.

It was known that leaf endogenous ABA concentration and stomatal behavior vary with species and are related to their adaptation to different water regimes. For example, some hygrophytic tree species usually grow in wet soils near watercourses and their stomatal conductance is reduced to a less extent when exposed to drought stress (Aasamaa and Sõber 2001). And these species had lower leaf ABA concentration (Aasamaa *et al*. 2002) and higher stomata conductivity (Loewenstein and Pallardy 1998, Aasamaa *et al*. 2002) than mesophytic tree species. On the contrary, stomatal behavior of young plants in some hemiepiphytic C_3 tree species was sensitive to water stress, since these species germinate and grow on another tree or rock, and thus, they may suffer from water deficits when their roots are not in direct contact with the soil (Holbrook and Putz 1996, Zotz and Hietz 2002).

From the reports mentioned above, it is known that ABA is an important chemical signal from roots which causes physiological changes in leaves, including stomata closure and photoprotection. Furthermore, endogenous ABA concentration in leaves and stomatal behavior vary with species and are related to their adaptation to different water regimes. In this study, *F. microcarpa*, a hemiepiphyte, *S. warburgii*, a hygrophyte, and *A. confusa*, a mesophyte, were used to elucidate the effects of osmotic- and high-light stresses on PSII efficiency of the attached and detached leaves.

Materials and methods

Plants: One- to two-year-old tree seedlings (about 40–60 cm high) from three species, *i.e*., *F. microcarpa* L., a hemiepiphytic C_3 tree, *S. warburgii* O. Seem., a hygrophyte, and *A. confusa* Merr., a mesophyte, were used. The former two species were propagated from cuttings, and *A. confusa* was propagated from seeds. They were planted in pots (16 cm diameter, 12 cm depth, one plant per pot) filled with sand and placed outdoor to receive regular water and fertilizers (1/2 strength of Hoagland's nutrient solution per month) and full sunlight on the campus of National Chung-Hsing University, Taichung, Taiwan (24°08'N, 120°40'E, 70 m a.s.l.). In addition, two months prior to the treatment of partial root-zone drying, the roots of one plant material of *F. microcarpa* and *S. warburgii* were allowed to grow into two plastic pots (16 cm diameter, 12 cm depth) which were taped together. In Taichung, mean monthly temperature, relative humidity and sunshine hours in 2005 were 16.1°C–29.0°C (Jan.–Aug.), 72%–84% (Dec.–Feb.) and 91.3 h–209.0 h (Jun.–Oct.), respectively (data from the Central Weather Bureau of Taiwan).

Comparison of Chl fluorescence, stomatal resistance and water potential (Ψ_w) of attached and detached leaves under osmotic- and high-light stresses: Experiments were carried out from September to October in 2005 to examine all three species mentioned above. At 17:00 h, shoots of *ca*. 20 cm lengths were cut from plants and immediately recut under water. Fully expanded upper leaf blade and petiole, detached shoot and intact plant were individually subjected to two levels of osmotic stress, created by different concentrations of mannitol solution (0.5 and 1.0 M for *F. microcarpa* and *S. warburgii* and 0.25 and 0.5 M for *S. warburgii*,

since the latter species is very sensitive to osmotic stress). Petioles of the detached leaves and bases of the detached shoots were inserted into mannitol solution or distilled water in test tubes, while plants with the attached leaves were irrigated with mannitol solution or water until the outflow appeared at the bottom of the pots. In addition, detached leaves of *F. microcarpa* also received ABA feeding treatment (100 μM ABA in 0.5 and 1.0 M of mannitol solutions). All materials were covered with plastic bags and put in the dark overnight with room temperature of *ca*. 25°C.

Measurements were made from 8:00 h in the next morning. Schedules of irradiance and the time course of measurements are shown in Fig. 1. First, Chl fluorescence of over-night dark-adapted upper, fully expanded leaves was measured. Subsequently, adaxial surfaces of the measured leaves were illuminated in sequence with 1,200 and 1,800 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) for 20 min and 120 min, respectively, by a slide projector with halogen light source. The Chl fluorescence of light-adapted leaves was measured at 20 min after the start of illumination with 1,200 μmol m^{-2} s^{-1} PPFD, and 60 and 120 min after the start of illumination with 1,800 μmol m^{-2} s^{-1} PPFD. Stomatal resistance was measured 30 min after 1,800 μmol m^{-2} s^{-1} PPFD illumination. Finally, materials were put in a dark room with a room temperature of *ca.* 25°C for 12 h. Ψ_w was measured 20 min after darkness. Chl fluorescence of dark-adapted leaves was measured at 20 min, 4 h, and 12 h after darkness.

PPFD was measured by a *LI-190SA* quantum sensor (*LI-COR*, Lincoln, NE, USA). Stomatal resistance was measured with a porometer (*AP-4, Delta-T Devices*, Burwell, Cambridge, UK). Ψ_w was measured by a thermocouple psychrometer (*C52* sample chambers connected to *HR33* dew-point microvolt meter, *Wescor*, Logan, Utah, USA). Chl fluorescence of both light- and dark-adapted leaves was measured with a portable pulse amplitude modulated fluorometer (*PAM-2000*, *Walz*, Effeltrich, Germany). The potential quantum efficiency of PSII (F_v/F_m) was calculated from $(F_m - F_0)/F_m$, and the actual PSII efficiency ($\Delta F/F_m'$) was calculated from $(F_m' - F)/F_m'$, respectively. F_0 and F_m, the minimal and maximal fluorescence in dark-adapted leaves, were determined by applying a weak pulse of red light [<0.1 μmol (quantum) m^{-2} s^{-1}] and a 1-s pulse of saturating flashes of approximately 6,000 μmol(quantum) m^{-2} s^{-1}, respectively. F and F_m' are the actual and the maximal levels of fluorescence during illumination, respectively. The former was determined under 1,200 or 1,800 μmol m^{-2} s^{-1} PPFD, and the latter was determined using the same process as for F_m.

Three to eleven leaves from 3 to 4 plants of each species were measured in each treatment. Each leaf was measured 3 (stomatal resistance and Ψ_w) to 5 (Chl fluorescence) times; and the mean of these measurements was taken as one replicate in statistical analyses.

Effects of osmotic stress on ABA accumulation in attached and detached leaves: *F. microcarpa* and *S. warburgii* were used for this treatment in October, 2005. Leaf detachment and osmotic stress were treated with the same methods as mentioned above. At 8:00 h of next morning, fully expanded younger leaves were harvested and rapidly stored at −80°C until use. The endogenous ABA, extracted from freeze-dried leaf samples by homogenization in 80% methanol, was purified and analyzed by gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM) using internal standards of [$^2H_6$]ABA (Chen *et al*. 2007). About 5 g of fresh leaves sampled from a plant was designated as a replicate, and 3 replicates were assigned to each treatment.

Effects of CO_2 diffusion restriction on Chl fluorescence: From September to October in 2005, attached leaves of *F. microcarpa* and *S. warburgii* received this treatment immediately before measurement by sealing the leaves with transparent films to prevent their gas exchange with the atmosphere (Haimeirong *et al*. 2002). Schedules of irradiance and the time course of measurements were the same as mentioned in the section of measurement of Chl fluorescence under osmotic- and high-light stresses. Five fully expanded upper leaves from 3 to 4 plants of each species were measured in each treatment. Each leaf was measured 5 times; and the mean of these measurements was taken as one replicate in statistical analyses.

Effects of partial root-zone drying on stomatal resistance and Ψ_w: *F. microcarpa* and *S. warburgii* were used for this treatment from September to October in 2005. The two plastic pots, in which the roots of one plant were allowed to grow into, received different watering regimes. While both pots for the control plants were watered to the drip point, only one pot for plants of partial root-zone drying treatment was similarly watered with the other pot receiving none. Stomatal resistance was measured around noontime 1−9, 16−18, and 22 days after treatment. In addition, Ψ_w was measured on the 1st and 22nd days of drying. Both parameters were measured with the same equipment and method as mentioned above. Fully expanded upper leaves from 4 plants of each treatment were measured, and the mean of 3 measurements from 3 leaves of one plant was taken as one replicate in statistical analyses.

Statistics: Data were analyzed by unpaired *t*-test, linear regression or *ANOVA* test. The former two were performed with *Sigma Plot* (version *9.01; Systat Software, Inc*., Point Richmond, CA, USA), and the latter was performed with *STATISTICA* software (version *6.0*; *Statsoft Inc.*, Tulsa, OK, USA).

Results

When leaves were exposed to light and recovered in the dark, the detached leaves of all three tested species had lower PSII efficiency than the attached leaves. Here *F. microcarpa* was selected as an example to illustrate (Fig. 1). Osmotic stress and detachment did not affect the potential efficiency of PSII (F_v/F_m value *ca.* 0.8) of all tested leaves before they were exposed to light. However, when the leaves were illuminated in sequence with 1,200 and 1,800 μmol m^{-2} s^{-1} PPFD for 20 min and 120 min, respectively, a pronounced decrease, *i.e.*, 16−30% as compared to prior-to illumination, of $\Delta F/F_m'$ value was observed. Subsequently, after 20 min in darkness, F_v/F_m of the attached leaves recovered to 77% (control), 69% (0.5 M mannitol-treated), and 65% (1 M mannitol-treated) of the value prior to illumination; and those F_v/F_m values of detached leaves were 32%, 24%, and 22% only, respectively. Following 12 h in darkness, F_v/F_m values recovered to 96% (control), 80% (0.5 M mannitol-treated) and 76% (1 M mannitol-treated) for attached leaves, and those of detached leaves only 50%, 37%, and 24%, respectively. From the above results, it is apparent that the maximum rate for the rising phase of F_v/F_m in darkness occurred in the initial 20 min after the light was turned off, and the F_v/F_m value at this time varied greatly among treatments. It is further illustrated in Fig. 2.

It shows that F_v/F_m of all three tested species, measured after illumination and dark-adapted for 20 min, was always negatively correlated with stomatal resistance and Ψ_w, except the cases mentioned below (Fig. 2). Among all three species, stomatal resistance of *F. microcarpa* was the most sensitive to osmotic stress, followed by *A. confusa* and *S. warburgii*. While Ψ_w of *F. microcarpa* was insensitive to osmotic stress, it was not related to F_v/F_m (Fig. 2*B*). On the contrary, that of both *A. confusa* and *S. warburgii* was sensitive to osmotic stress and showed a negative correlation with F_v/F_m. However, due to the very low F_v/F_m in detached *S. warburgii* leaves, both F_v/F_m-stomatal resistance and F_v/F_m-Ψ_w correlations were insignificant (Fig. 2*E*,*F*). Compared at the same levels of stomatal resistance and

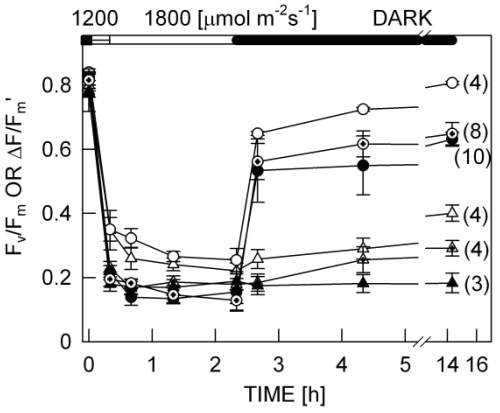

Fig. 1. Time course of illumination (1,200 μmol m^{-2} s^{-1} PPFD for 20 min and then 1,800 μmol m^{-2} s^{-1} PPFD for 120 min) and darkness (12 h); and PSII efficiency (F_v/F_m and $\Delta F/F_m'$) of osmotic-stressed and control *Ficus microcarpa* leaves under illumination and darkness. Values are means ± SE; numeric value within the parentheses are sample size of each treatment; ○ and Δ: no osmotic stress; ⊕ and ⧋: 0.5 M mannitol; ● and ▲: 1.0 M mannitol; *circle* and *triangle symbols*: attached and detached leaves, respectively.

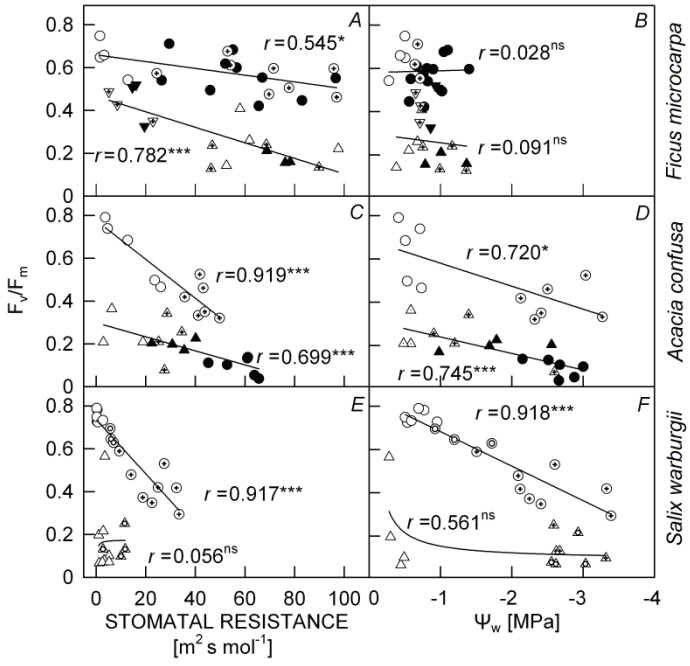

Fig. 2. Under osmotic- and high-light (1,200 μmol m^{-2} s^{-1} PPFD for 20 min and then 1,800 μmol m^{-2} s^{-1} PPFD for 120 min) stresses, F_v/F_m of *Ficus microcarpa*, *Acacia confusa* and *Salix warburgii* as affected by stomatal resistance and leaf water potential (Ψ_w). Each point represents the mean value of 1 leaf. ○ and Δ: no osmotic stress; ◎ and ⧋: 0.25 M mannitol; ⊕, ⧋ and ⧩: 0.5 M mannitol; ●, ▲ and ▼: 1.0 M mannitol; *circle symbols*: attached leaves; *triangle up* and *down symbols*: detached leaves, cut at the base of petiole and shoot, respectively; each regression line was grouping of the data obtained from attached or detached leaves, except severe osmotic stress exposed *A. confusa* attached leaves (● in panels *C* and *D*, it was grouping to detached leaves); ***, * and ns: $p<0.001$, $p<0.05$ and no significant, respectively.

Table 1. ABA concentration of the attached and detached *Ficus microcarpa* and *Salix warburgii* leaves under osmotic stress (0.5 M mannitol) or water. Values are means ± SE [n = 3 (for the attached *F. microcarpa* leaves under 0.5 M mannitol) to 4 (for the other)], and within a row followed by the same characters do not differ significantly (p>0.05) according to *ANOVA* test.

Species	ABA concentration [nmol g^{-1}(DM)] Mannitol		Water	
	Attached	Detached	Attached	Detached
F. microcarpa	166.6 ± 9.8[a]	123.1 ± 3.1[b]	115.5 ± 5.6[b]	-
S. warburgii	0.211 ± 0.012[c]	0.646 ± 0.046[d]	0.162 ± 0.017[c]	0.211 ± 0.028[c]

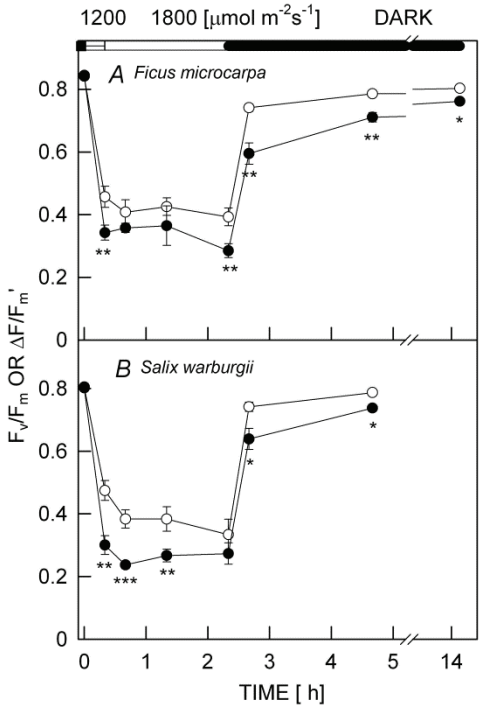

Fig. 3. Time course of illumination (1,200 μmol m^{-2} s^{-1} PPFD for 20 min and then 1,800 μmol m^{-2} s^{-1} PPFD for 120 min) and darkness (12 h); PSII efficiency (F_v/F_m and $\Delta F/F_m$') of control (○) and CO_2 diffusion-limited attached (●) *Ficus microcarpa* (*A*) and *Salix warburgii* (*B*) leaves under illumination and darkness. Each point represents the mean value of 5 leaves; and values given are means ± SE. *, ** and ***: Significant differences between control and CO_2 diffusion-limited leaves at p<0.05, p<0.01 and p<0.001, respectively, based on unpaired t-test.

Ψ_w, the attached *F. microcarpa* leaves showed the highest F_v/F_m, followed by *A. confusa* and *S. warburgii*. For the detached leaves, either treated with two levels of osmotic stress or not, F_v/F_m was lower than that of the attached leaves for all three tested species. However, attached *A. confusa* leaves in 1 M mannitol, which showed lower F_v/F_m value, could be grouped together with the detached leaves (Fig. 2*C,D*).

It shows that *F. microcarpa* leaves, even in well watered condition, contained higher level of endogenous ABA, and osmotic stress could raise it in the attached leaves but not in the detached ones of this plant (Table 1). On the contrary, the endogenous ABA concentration of the attached *S. warburgii* leaves was very low and not affected by osmotic stress; however, under such stress, ABA concentration in the detached leaves increased. It shows that, for attached leaves of both *F. microcarpa* and *S. warburgii*, CO_2 limitation not only enhanced the decline of $\Delta F/F_m$' under illumination, but also decreased the recovery of F_v/F_m in the dark (Fig. 3). Under the osmotic stress of 0.5 M mannitol, stomatal resistance of ABA-treated *F. microcarpa* leaves was significantly higher than that of the nontreated leaves; but there was no significant difference in F_v/F_m between them (Fig. 4*B*). On the contrary, both ABA-treated and nontreated *F. microcarpa* leaves showed high level of stomatal resistance, and ABA could mitigate the decrease of F_v/F_m in detached leaves of this tree under severe (1 M mannitol) osmotic stress. It also indicates that in the absence of ABA treatment, F_v/F_m decreased with the increase of stomatal resistance, when data obtained from the two levels of osmotic stress were merged (Fig. 4*A*). On the contrary, F_v/F_m values of all ABA-treated leaves were higher than those of the regression line obtained from the leaves receiving none of this plant hormone, indicating that ABA-treated leaves could maintain a higher level of F_v/F_m even when stomata closure was enhanced.

Ψ_w was not affected by partial root-zone drying treatment for both two tested species. However, stomatal resistance of *F. microcarpa* increased *ca.* 10 days after treatment, and *S. warburgii* maintained a low stomatal resistance until the end of experiment, *i.e.*, 22 days after treatment (Fig. 5).

Discussion

Osmotic- and high-light stresses often led to photoinhibition because the leaf absorbed light energy in excess of the amount it can utilize for photosynthesis (Stuhlfauth *et al.* 1990, Valladares and Pearcy 1997). Results of the present study indicate that photoinhibition occurred under osmotic- and high-light stresses, and yet, this inhibition varied with leaf detachment. $\Delta F/F_m$', the actual PSII efficiency under illumination, of the osmotic-stressed leaves decreased significantly when the leaves were subsequently exposed to light; and when the light

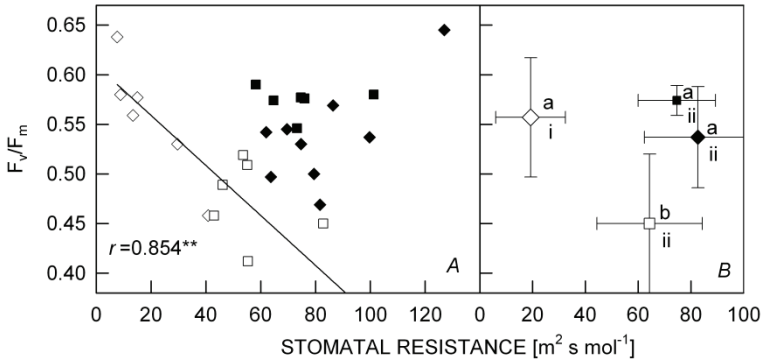

Fig. 4. Relationship between F_v/F_m and stomatal resistance of detached *Ficus microcarpa* leaves under osmotic- and high-light (1,200 μmol m^{-2} s^{-1} PPFD for 20 min and then 1,800 μmol m^{-2} s^{-1} PPFD for 120 min) stresses, with and without ABA. *A*: each point represents the value of 1 leaf; *B*: averaged values on *A* (means ± SD); *diamond* and *square symbols*: 0.5 M and 1.0 M mannitol, respectively; *closed* and *open symbols*: with and without ABA (100 μM) treatment, respectively; a *vs*. b and i *vs*. ii: different characters represent significant difference (p<0.05) for F_v/F_m and stomatal resistance, respectively, based on *ANOVA* test; **: p<0.01.

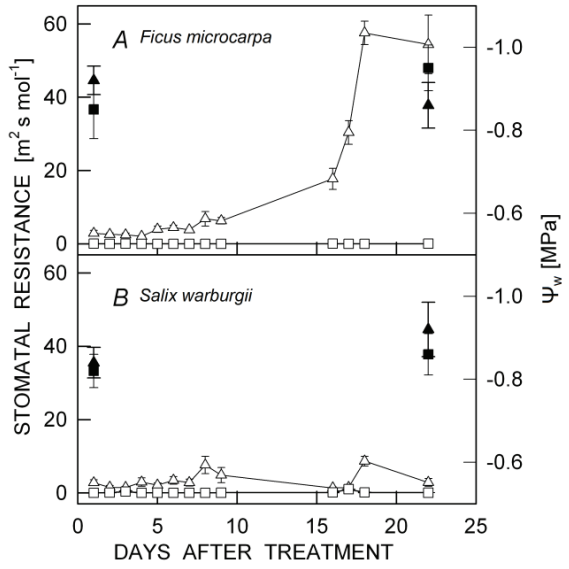

Fig. 5. Stomatal resistance (*open symbols*) and leaf water potential (Ψ_w) (*closed symbols*) of *Ficus microcarpa* and *Salix warburgii* in well-watered control (*square symbols*) and partial root-zone drying (*triangle symbols*) treatments. Each point represents the mean value of 4 plants; values are means ± SE.

was turned off for 20 min, F_v/F_m, the potential PSII efficiency, could reverse to a certain extent, and yet failed to regain the level prior to illumination (Fig. 1). Such a decrease of the slope of the rising phase of F_v/F_m has been interpreted as a reflection of damage to plant PSII (Potvin 1985, Maxwell and Johnson 2000). As shown in Figs. 1 and 2, after illumination and subsequent dark-adaptation for 20 min, F_v/F_m decreased with increasing osmotic stress, namely, decreasing Ψ_w or increasing stomatal resistance. However, when compared at the same level of Ψ_w or stomatal resistance, F_v/F_m of detached leaves, excised from both the base of the petiole and the base of the shoot, was lower than that of leaves attached to the plants for all three tree species studied in this work. These results indicate that, under osmotic- and high-light stresses, a more drastic photoinhibition was induced in the detached leaves than in the attached ones, in spite of the fact that the tested species are adapted to different water regimes, and difference in physiological responses to osmotic stress.

Potvin (1985) suggested that water loss might be a problem in detached or excised leaves. Results of the present study show that Ψ_w of *F. microcarpa* was insensitive to two levels of osmotic stress, and no significant difference in Ψ_w was detected among treatments. Nevertheless, F_v/F_m of the detached *F. microcarpa* leaves was still lower than that of the attached ones (Fig. 2*B*). On the contrary, Ψ_w of *S. warburgii* leaves was very sensitive to osmotic stress in both the attached and detached leaves, with that of *A. confusa* to osmotic stress falling in between. Despite the fact that F_v/F_m of *S. warburgii* and *A. confusa* leaves decreased with decreasing Ψ_w, detached leaves showed lower F_v/F_m than the attached leaves when compared at the same level of Ψ_w (Fig. 2*D,F*). From the above results, it was concluded that water loss was not a reason for a low F_v/F_m in detached leaves. Since F_v/F_m is widely used as a reliable diagnostic indicator of photoinhibition (Maxwell and Johnson 2000), and the latter is often enhanced due to the limitation of CO_2 diffusion to the chloroplast (Kato *et al*. 2002, Murata *et al*. 2007). Results of the present study also indicate that osmotic stress could enhance stomatal closure (Fig. 2). Yet, limited CO_2 diffusion could reduce F_v/F_m (Fig. 3). Even though F_v/F_m showed a negative correlation with leaf stomatal resistance, the detached leaves still showed lower F_v/F_m than the attached leaves for all the three species when compared at the same level of leaf stomatal resistance (Fig. 2*A*,*C*,*E*). Therefore, limited CO_2 diffusion was not a reason for a low F_v/F_m in excised leaves.

What would be the possible causes for the higher sensitivity of PSII to osmotic- and high-light stresses in detached leaves than in attached ones? One might be due to the root-sourced signals. It is well known that, under

water deficit or osmotic stresses, ABA is an important root-to-shoot stress signal to modify stomatal behavior (Dodd 2005, Mancuso and Mugnai 2006, Jia and Zhang 2008, Dodd *et al*. 2009). Even with only a part of roots exposed to drying soil and nonhydraulic limitation in shoots, stomatal conductance and leaf growth could be regulated by signals from drying roots (Davies and Zhang 1991, Dodd 2005, Jia and Zhang 2008). In addition, ABA could also play a role in protecting PSII against the damaging effects of excess absorbed light energy (Beckett *et al*. 2000, Jiang and Zhang 2001, Sharma *et al*. 2002, Jia and Lu 2003, Agarwal *et al*. 2005, Lu *et al*. 2009). In the present study, we used three tree species with different sensitivity of stomatal behavior and Ψ_w towards osmotic stress. Among them, *F. microcarpa*, a hemiepiphytic C_3 tree species, has been generally considered as drought-insensitive plant, while *S. warburgii*, usually growing in wet soil near watercourse, is generally considered as drought-sensitive. Results indicate that the leaves of *F. microcarpa* contained higher level of endogenous ABA (Table 1), and its stomatal resistance was sensitive to osmotic stress (Fig. 2*A*) as well as partial root-zone drying treatment (Fig. 5). On the contrary, leaves of *S. warburgii* contained very low level of endogenous ABA (Table 1), and its stomatal resistance was insensitive to either osmotic stress or partial root-zone drying treatment (Figs. 2*E*, 5). Fig. 2 also shows that, when compared at the same levels of osmotic- and high-light stresses, attached *F. microcarpa* leaves showed the highest F_v/F_m, followed by *A. confusa* and *S. warburgii*. These results generally agreed with the results of water relation, ABA content, and stomata behavior obtained from hygrophytic (Loewenstein and Pallardy 1998, Aasamaa and Sõber 2001, Aasamaa *et al*. 2002) and hemiepiphytic (Holbrook and Putz 1996, Zotz and Hietz 2002) tree species. These species-specific differences could be explained by its capability to maintain the balance of CO_2 uptake/water loss under different water regime.

In order to enhance the effects of irradiation on photoinhibition both the attached and detached leaves were exposed to 1,200 μmol m^{-2} s^{-1} PPFD for 20 min and then to 1,800 μmol m^{-2} s^{-1} PPFD for 120 min. For the detached leaves, other factors (*e.g*. restricted assimilate phloem transport, shortage of nutrients needed to run reparation cycles) might also be involved in affecting the response during this time period. Nevertheless, Fig. 4 shows that ABA-treated, detached *F. microcarpa* leaves could maintain a higher level of F_v/F_m under severe (1 M mannitol) osmotic- and high-light stresses, even when stomata closure was enhanced. This result indicates that ABA may act by maintaining the PSII efficiency of detached *F. microcarpa* leaves. On the contrary, there was no significant difference in F_v/F_m between ABA-treated and nontreated detached *F. microcarpa* leaves under 0.5 M mannitol osmotic stress (Fig. 4*B*). Because stomatal resistance of ABA-treated *F. microcarpa* leaves was significantly higher than that of nontreated leaves under 0.5 M mannitol osmotic stress (Fig. 4*B*), the limited CO_2 diffusion could have reduced F_v/F_m (Fig. 3). Therefore, it is proposed that the protecting effect of ABA on F_v/F_m might be offset by a CO_2 limitation due to stomatal closure under 0.5 M mannitol. Results of the present study also indicate that partial root-zone drying exerted a significant effect on the stomatal behavior of *F. microcarpa* leaves (Fig. 5*A*), and ABA concentration increased in attached *F. microcarpa* leaves when the roots were exposed to osmotic stress (Table 1). Therefore, it was probable that, for *F. microcarpa*, the higher PSII efficiency of attached leaves under osmotic- and high-light stresses might be related to the protection by ABA transported from osmotically stressed roots.

However, a completely opposite phenomenon was observed for *S. warburgii* in the present study. Osmotic stress did not affect the concentration of leaf endogenous ABA in the attached leaves, but increased it in the detached leaves. Nevertheless, *S. warburgii* contained only a very low level of endogenous ABA (Table 1). Moreover, its stomatal behavior was not influenced by partial root-zone drying (Fig. 5*B*). Because lower leaf ABA concentration and higher stomatal opening were also found in another hygrophyte *Salix caprea* (Aasamaa *et al*. 2002), it is clear that the higher PSII efficiency in the attached leaves of *S. warburgii* under osmotic- and high-light stresses could not be attributed to the protection by ABA transported from osmotically stressed roots. It has been reported that the other types of stress signals could be sent out from roots (Dodd 2005, Mancuso and Mugnai 2006, Dong *et al*. 2008, Jia and Zhang 2008). Therefore, these signals might have a role in protecting PSII against the damaging effects of excess absorbed energy in attached *S. warburgii*, probably even in *F. microcarpa* leaves. However, these signals were not examined in this study, it would be deserved further study. In addition, based on the data obtained in the present study, we could not explain why *A. confusa* attached leaves, which had been exposed to severe osmotic stress prior to high-light stress, showed tendency of F_v/F_m similar to those of the detached leaves (Fig. 2*C,D*). Further experiments are needed to be conducted to provide the explanation.

From the above results it is evident that, under osmotic- and high-light stresses, PSII efficiency would decrease with increasing stomatal closure and water loss. However, at the same levels of stomatal resistance and leaf water potential, detachment of leaves either at the base of the petiole or the shoot would decrease their PSII efficiency. This lower efficiency for PSII of the detached leaves might be linked to the plant hormone ABA or other signals from the root system. It is suggested that the detached leaves are not suitable for the research of water or osmotic stress due to the loss of the signals from the roots.

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