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

J.-H. WENG[*, ***, +], C.-T. CHIEN[**], C.-W. CHEN[***], and X.-M. LAI[***]

*Graduate Institute of Ecology and Evolutionary Biology, China Medical University, Taichung, Taiwan**

*Division of Silviculture, Taiwan Forestry Research Institute, Taipei, Taiwan***

*Department of Life Science, National Chung-Hsing University, Taichung, Taiwan****

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 water adaptability of different species. In this study, *Ficus microcarpa*, a hemiepiphyte, *Salix warburgi*, 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 detached leaves was lower than that of 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 attached, but not in detached *F. microcarpa* leaves. In addition, partial root-zone drying exerted a significant effect on the stomatal behavior, but not on water status of *F. microcarpa* leaves. These observations imply that the stronger ability of PSII in 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: ABA; *Acacia confusa*; chlorophyll fluorescence; *Ficus microcarpa*; osmotic stress; *Salix warburgii*.

+Author for correspondence; fax: +886 4 22071507, e-mail: jhweng@mail.cmu.edu.tw

Abbreviations: ABA – abscisic acid; 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.

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 photosystem II (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).

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 non-hydraulic 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, abscisic acid (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 xanthophylls 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 its 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 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 fluorescence values of detached leaves from 4 species were lower than those of attached leaves. On the contrary, Percival and Fraser (2001) did not detect any detrimental effects on chlorophyll fluorescence values when the leaves were assessed 72 hours 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 water adaptability. For example, stomata of some hygrophytic tree species, which usually grow in wet soils near watercourses, were found insensitive to water 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, 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 water adaptability. In this study, *Ficus microcarpa*, a hemiepiphyte, *Salix warburgi*, a hygrophyte, and *Acacia confusa*, a mesophyte,

were used to elucidate the effects of osmotic and high light stresses on PSII efficiency of attached and detached trees leaves.

Materials and Methods

Materials: One- to two-year-old tree seedlings (about 40-60 cm height) from three species, *i.e.*, *Ficus microcarpa* L., a hemiepiphytic C_3 tree, *Salix warburgii* O. Seem., a hygrophyte, and *Acacia 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^o 08' N, 120^o 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 hour in 2005 were 16.1°C (Jan.)-29.0°C (Aug.), 72% (Dec.)-84% (Feb.) and 91.3 h (Jun.)-209.0 h (Oct.), respectively (data from the Central Weather Bureau of Taiwan).

Comparison of chlorophyll fluorescence, stomatal resistance and water potential 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 re-cut 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

127 concentrations of mannitol solution (0.5 and 1.0 M for *F. microcarpa* and *S. warburgii* and
128 0.25 and 0.5 M for *S. warburgii*, since the latter species is very sensitive to osmotic stress).
129 Petioles of detached leaves and bases of detached shoots were inserted into mannitol solution
130 or distilled water in test tubes, while plants with attached leaves were irrigated with mannitol
131 solution or water until the outflow appeared at the bottom of the pots. In addition, detached
132 leaves of *F. microcarpa* also received ABA feeding treatment (100 μM ABA in 0.5 and 1.0
133 M of mannitol solutions). All materials were covered with plastic bags and put in the dark
134 overnight with room temperature of *ca*. 25°C.

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

147 PPFD was measured by a *LI-190SA* quantum sensor (*LI-COR*, Lincoln, NE, USA).
148 Stomatal resistance was measured with a porometer (*AP-4*, *Delta-T Devices*, Burwell**,**
149 Cambridge, UK). Leaf water potential was measured by a thermocouple psychrometer
150 (*C52* sample chambers connected to *HR33* dew-point microvolt meter, *Wescor*, Logan,

Utah, USA). Chlorophyll 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 quanta m^{-2} s^{-1}) and a 1-s pulse of saturating flashes of approximately 6 000 μmol quanta 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 11 leaves from 3 to 4 plants of each species were measured in each treatment. Each leaf was measured 3 (stomatal resistance and water potential) to 5 (chlorophyll 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 chlorophyll 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 change 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 chlorophyll 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 water potential: *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 the 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, leaf water potential was measured on the 1^{st} and 22^{nd} 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

199 former two were performed with Sigma Plot (*version 9.01; Systat Software, Inc*., Point
200 Richmond, CA, USA), and the latter was performed with *STATISTICA* software (*version 6.0*;
201 *Statsoft* Inc, Tulsa, OK, USA).
202
203 **Results**
204 The three tested species demonstrated a similar response on PSII efficiency between attached
205 and detached leaves when they exposed to light and recovered in the dark. Here *F.*
206 *microcarpa* (Fig. 1) was selected as an example to illustrate. Osmotic stress and detachment
207 did not affect the potential efficiency of PSII (F_v/F_m value *ca.* 0.8) of all tested leaves
208 before they were exposed to light. However, when the leaves were illuminated in sequence
209 with 1 200 and 1 800 μmol m^{-2} s^{-1} PPFD for 20 min and 120 min, respectively, a pronounced
210 decrease, *i.e.*, 16-30% as compared to prior-to illumination, of $\Delta F/F_m$' value was observed.
211 Subsequently, after 20 min in darkness, F_v/F_m of attached leaves recovered to 77% (control),
212 69% (0.5 M mannitol-treated) and 65% (1 M mannitol-treated) of the value prior to
213 illumination; and those F_v/F_m values of detached leaves were 32%, 24% and 22% only,
214 respectively. Following 12 hours in darkness, F_v/F_m values recovered to 96% (control), 80%
215 (0.5 M mannitol-treated) and 76% (1 M mannitol-treated) for attached leaves, and those of
216 detached leaves only 50%, 37% and 24%, respectively. From the above results, it is apparent
217 that the maximum rate for the rising phase of F_v/F_m in darkness occurred in the initial 20 min
218 after the light was turned off, and the F_v/F_m value at this time varied greatly among
219 treatments. It is further illustrated in Fig. 2.
220 It shows that F_v/F_m of all three tested species, measured after illumination and
221 dark-adapted for 20 min, was always negatively correlated with stomatal resistance and leaf
222 water potential, 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 leaf water potential 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-leaf water potential correlations were insignificant (Fig. 2*E*,*F*). Compared at the same levels of stomatal resistance and leaf water potential, 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 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 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 attached, but not in detached, leaves of this plant (Table 1). On the contrary, the endogenous ABA concentration of attached *S. warburgii* leaves was very low, and not affected by osmotic stress; however, under such stress, ABA concentration in detached leaves was 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 non-treated leaves; but there was no significant difference in F_v/F_m between them (Fig. 4*B*). On the contrary, both ABA-treated and non-treated *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 the absence of ABA

248 treatment, F_v/F_m decreased with the increase of stomatal resistance, when data obtained from
249 the two levels of osmotic stress were merged (Fig. 4A). On the contrary, F_v/F_m values of all
250 ABA-treated leaves were higher than those of the regression line obtained from the leaves
251 receiving none of this plant hormone, indicating that ABA-treated leaves could maintain a
252 higher level of F_v/F_m even when stomata closure was enhanced.

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

257

258 ## Discussion

259 Osmotic and high light stresses often led to photoinhibition because the leaf absorbed light
260 energy in excess of the amount it can utilize for photosynthesis (Stuhlfauth *et al*. 1990,
261 Valladares and Pearcy 1997). Results of the present study indicate that photoinhibition
262 occurred under osmotic and high light stresses, and yet, this inhibition varied with leaf
263 detachment. $\Delta F/F_m$', the actual PSII efficiency under illumination, of the osmotic-stressed
264 leaves decreased significantly when the leaves were subsequently exposed to light; and
265 when the light was turned off for 20 min, F_v/F_m, the potential PSII efficiency, could reverse
266 to a certain extent, and yet failed to regain the level prior to illumination (Fig. 1). Such a
267 decrease of the slope of the rising phase of F_v/F_m has been interpreted as a reflection of
268 damage to plant PSII (Potvin 1985, Maxwell and Johnson 2000). As shown in Figs. 1 and 2,
269 after illumination and subsequent dark-adaptation for 20 min, F_v/F_m decreased with
270 increasing osmotic stress, namely, decreasing leaf water potential or increasing stomatal
271 resistance. However, when compared at the same level of leaf water potential or stomatal
272 resistance, F_v/F_m of detached leaves, excised from both the base of the petiole and the base of

273 the shoot, was lower than that of leaves attached to the plants for all three tree species
274 studied in this work. These results indicate that, under osmotic and high light stresses, a more
275 drastic photoinhibition was induced in detached leaves than in attached leaves, in spite of the
276 fact that tested species are adapted to different water regimes, and difference in physiological
277 responses to osmotic stress.

278 Potvin (1985) suggested that water loss might be a problem in detached or excised
279 leaves. Results of the present study show that leaf water potential of *F. microcarpa* was
280 insensitive to two levels of osmotic stress, and no significant difference in leaf water
281 potential was detected among treatments. Nevertheless, F_v/F_m of detached *F. microcarpa*
282 leaves was still lower than that of attached leaves (Fig. 2*B*). On the contrary, water potential
283 of *S. warburgii* leaves was very sensitive to osmotic stress in both attached and detached
284 leaves, with that of *A. confusa* to osmotic stress falling in between. Despite of the fact that
285 F_v/F_m of *S. warburgii* and *A. confusa* leaves decreased with decreasing leaf water potential,
286 detached leaves showed lower F_v/F_m than attached leaves when compared at the same level
287 of leaf water potential (Fig. 2*D*,*F*). From the above results, it was concluded that water loss
288 was not a reason for a low F_v/F_m in detached leaves. Photoinhibition was often enhanced due
289 to the limitation of CO_2 diffusion to the chloroplast (Kato *et al*. 2002, Murata *et al*. 2007).
290 Results of the present study also indicate that osmotic stress could enhance stomatal closure
291 (Fig. 2). Yet, limited CO_2 diffusion could reduce F_v/F_m (Fig. 3). Even though F_v/F_m showed
292 a negative correlation with leaf stomatal resistance, detached leaf still showed lower F_v/F_m
293 than attached leaf for all the three species when compared at the same level of leaf stomatal
294 resistance (Fig. 2*A*,*C*,*E*). Therefore, limited CO_2 diffusion was not a reason for a low F_v/F_m
295 in excised leaves.

296 What would be the possible causes for the higher sensitivity of PSII to osmotic and
297 high light stresses in detached leaves than in attached leaves? One might be due to the

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

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

343 However, a completely opposite phenomenon was observed for *S. warburgii* in the
344 present study. Osmotic stress did not affect the concentration of leaf endogenous ABA in
345 attached leaves, but increased it in detached leaves. Nevertheless, *S. warburgii* contained
346 only a very low level of endogenous ABA (Table 1). Moreover, its stomatal behavior was not
347 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 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 in their PSII efficiency. This lower efficiency for PSII of detached leaves might be linked to 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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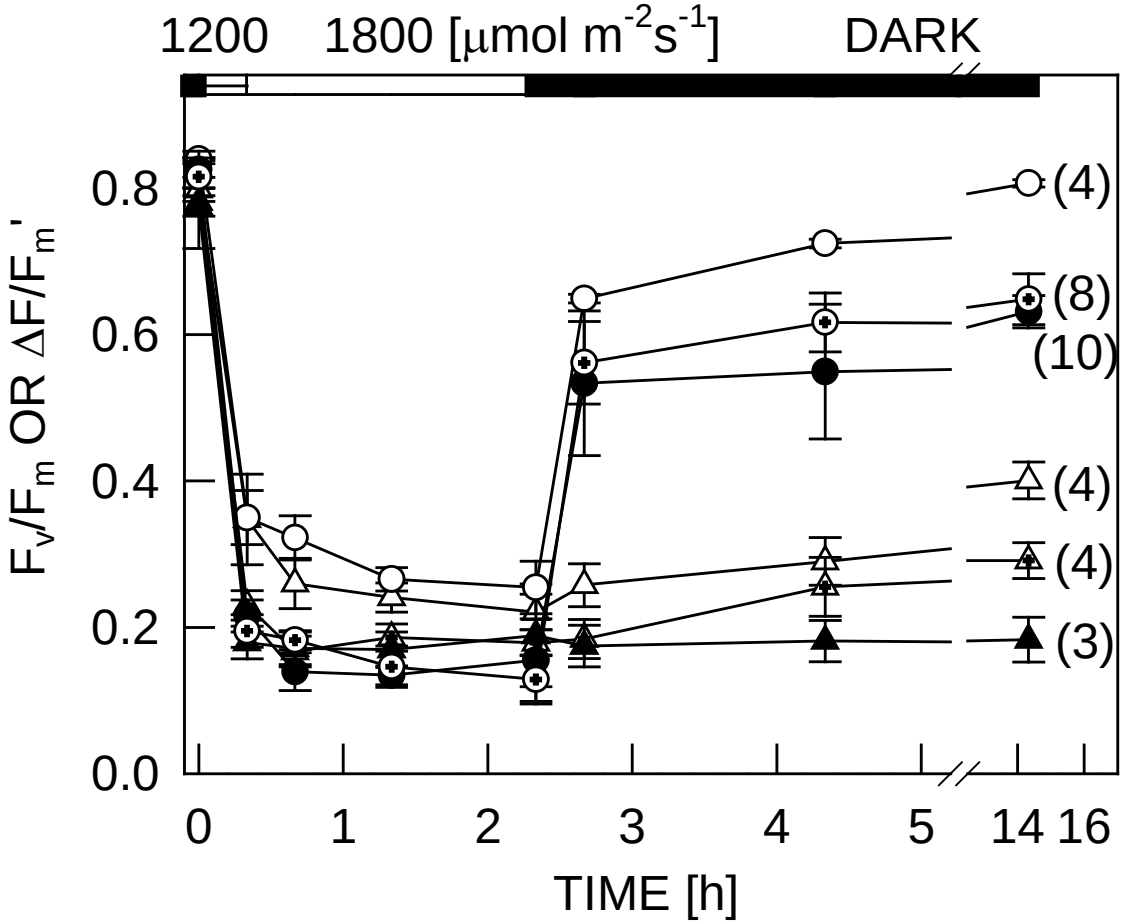
472 Zotz, G., Hietz, P.: The physiological ecology of vascular epiphytes: current knowledge,
473 open questions. – J. Exp. Bot. **52:** 2067-2078, 2002.
474

474 Table 1. ABA concentration (ng g^{-1}) of attached and detached *Ficus microcarpa* and *Salix*
475 *warburgii* leaves under osmotic (0.5 M mannitol) or no-osmotic (water) stress. Values are
476 means ± SE [*n*=3 (for attached *F. microcarpa* leaves under 0.5 M mannitol) to 4 (for the
477 other)], and within a row followed by the same characters do not differ significantly
478 (p>0.05) according to *ANOVA* test.

479

Species	Mannitol		Water	
	Attached	Detached	Attached	Detached
Ficus microcarpa	166.6±9.8[a]	123.1±3.1[b]	115.5±5.6[b]	---
Salix warburgii	0.211±0.012[c]	0.646±0.046[d]	0.162±0.017[c]	0.211±0.028[c]

480



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482 Fig. 1. Time course of illumination (1 200 μmol m^{-2} s^{-1} PPFD for 20 min and then 1 800

483 μmol m^{-2} s^{-1} PPFD for 120 min) and darkness (12 h); and PSII efficiency (F_v/F_m and $\Delta F/F_m$')

484 of osmotic-stressed and control *Ficus microcarpa* leaves under illumination and darkness.

485 Values are means ± SE; numeric value within the parentheses are sample size of each

486 treatment; ○ and △: no osmotic stress; ⊕ and ⛬: 0.5 M mannitol; ● and ▲: 1.0 M

487 mannitol; *circle* and *triangle symbols*: attached and detached leaves, respectively.

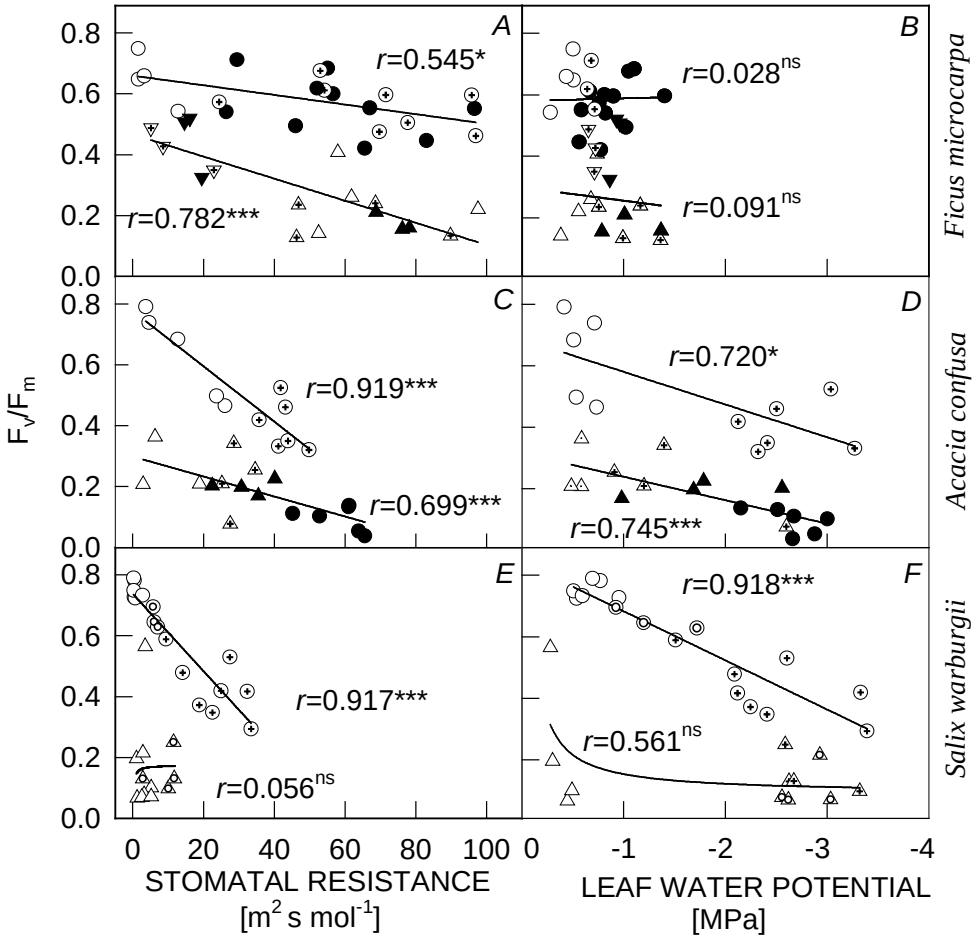


Fig. 2. Under osmotic and high light (1 200 μmol m^{-2} s^{-1} PPFD for 20 min and then 1 800 μmol m^{-2} s^{-1} PPFD for 120 min) stresses, F_v/F_m of *Ficus microcarpa*, *Acacia confusa* and *Salix warburgii* as affected by stomatal resistance and leaf water potential. Each point represents the mean value of 1 leaf. ○ and △: 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.

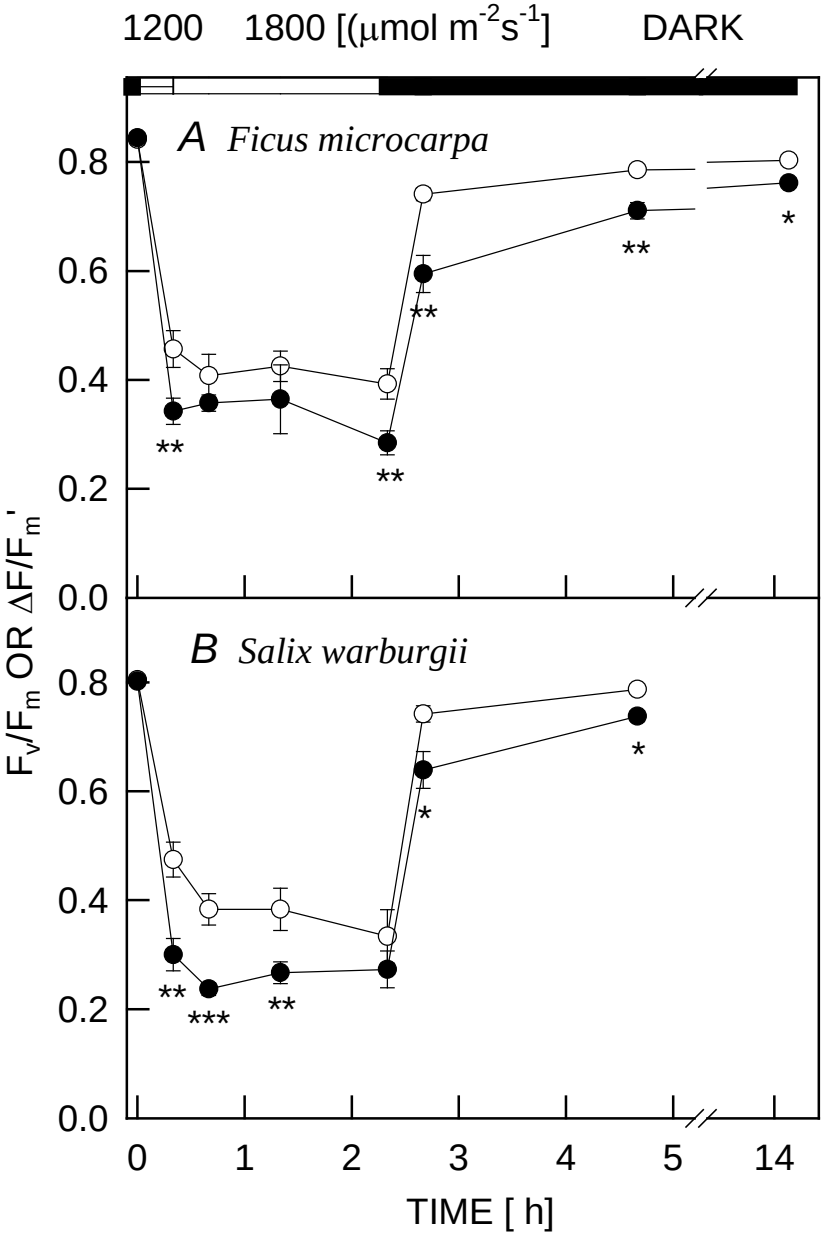
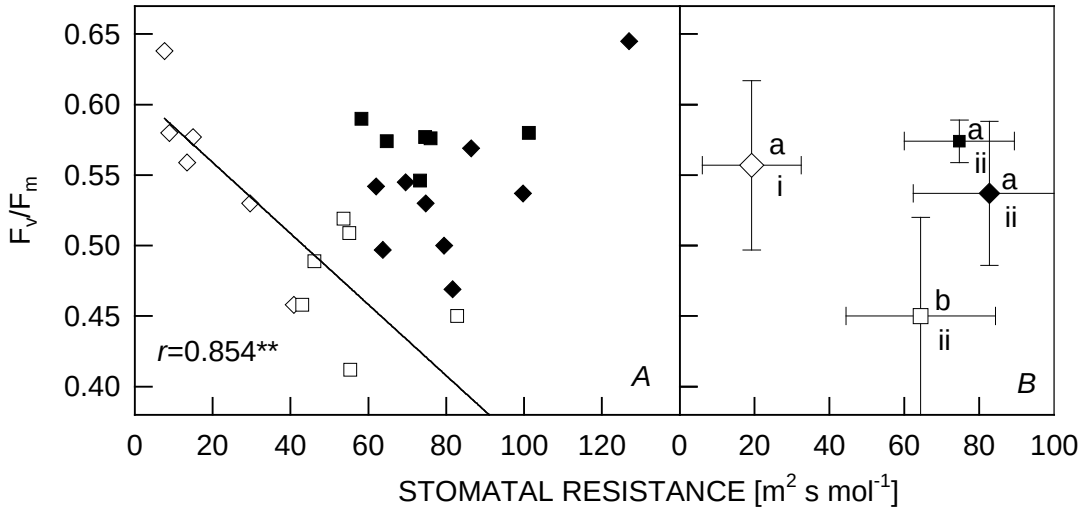


Fig. 3. Time course of illumination (1 200 μmol m^{-2} s^{-1} PPFD for 20 min and then 1 800 μmol m^{-2} s^{-1} PPFD for 120 min) and darkness (12 hours); PSII efficiency (F_v/F_m and $\Delta F/F_m'$) of control (○) and CO_2 diffusion-limited attached (●) *Ficus microcarpa* (*A*) and *Salix warburgii* (*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.



509

510 Fig. 4. Relationship between F_v/F_m and stomatal resistance of detached *Ficus microcarpa*
511 leaves under osmotic and high light (1 200 μmol m^{-2} s^{-1} PPFD for 20 min and then 1 800
512 μmol m^{-2} s^{-1} PPFD for 120 min) stresses, with and without ABA. *A*: each point represents
513 the value of 1 leaf; *B*: averaged values on *A* (means ± SD); *diamond* and *square symbols*:
514 0.5 M and 1.0 M mannitol, respectively; *close* and *open symbols*: with and without ABA
515 (100 μM) treatment, respectively; a vs. b and i vs. ii: different characters represent
516 significant difference ($p < 0.05$) for F_v/F_m and stomatal resistance, respectively, based on
517 *ANOVA* test; **: $p < 0.01$.

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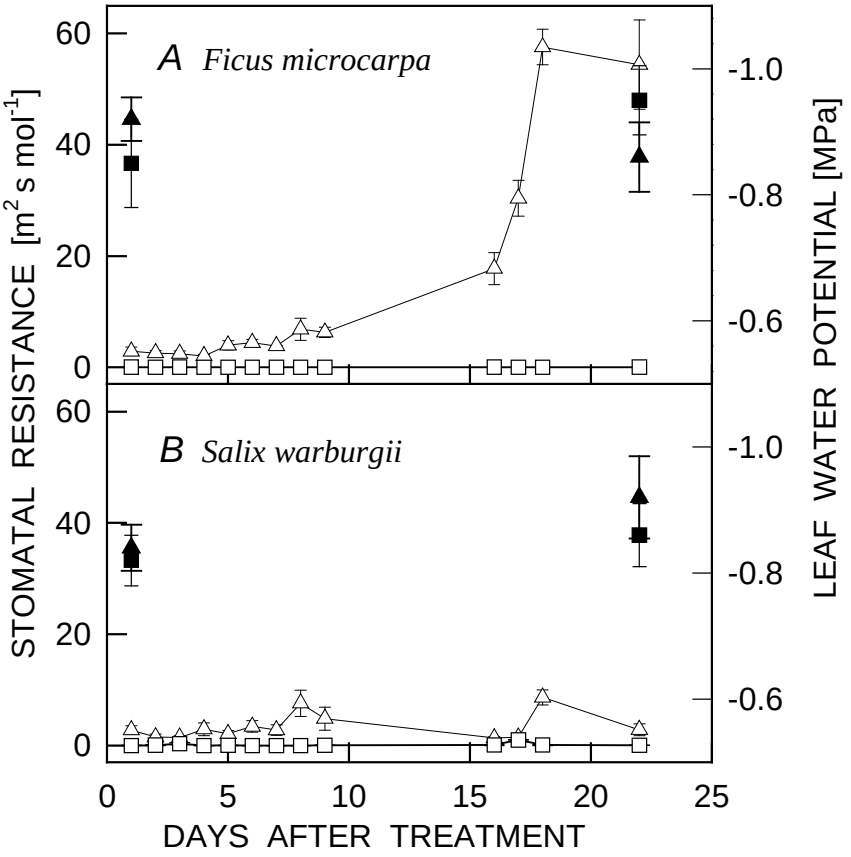

519 Fig. 5. Stomatal resistance (*open symbols*) and leaf water potential (*close symbols*) of *Ficus*
520 *microcarpa* and *Salix warburgii* in well-watered control (*square symbols*) and partial
521 root-zone drying (*triangle symbols*) treatments. Each point represents the mean value of 4
522 plants; values are means ± SE.
523