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# Responses of Photosystem II (PSII) Function in Leaves and Samaras of *Ulmus pumila* to Chilling and Freezing Temperatures and Subsequent Recovery

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## ABSTRACT

The responses of photosystem II (PSII) activity of samara and leaf of Siberian elm (*Ulmus pumila* L.) to chilling (5°C) and freezing (-5°C & -15°C) temperature and their recovery were investigated. There was little difference in Fv/Fm between samara and leaf. Leaf showed more efficient photosynthesis ($PI_{ABS}$) and electron transport ($\varphi_{Eo}$ & $\Psi o$) than samara. Chilling and freezing had adverse impact on PSII function in both samara and leaf. Low temperature stress decreased photochemical efficiency (Fv/Fm & $PI_{ABS}$) and electron transport activity ($\psi_o$, $\varphi_{Eo}$ & $ET_o/RC$) in both leaf and samara. PSII of samara was more tolerant to low temperature stress than leaf. Photochemical efficiency (Fv/Fm) for chilled (at 5°C) or mild frozen (at -5°C) samara or leaf showed a recovery trend. Severe freezing stress (-15°C) led to drastic and irreversible injuries to PSII in samara and leaf. The fact that samara has photosynthetic activity and is more tolerant to low temperature than leaf is of great ecological significance for seed development, population establishment and the northern distribution limit of Siberian elm in northern hemisphere. © 2011 Friends Science Publishers

**Key Words:** Chilling; Freezing; Samara; Chlorophyll fluorescence; Photosystem II; JIP-test analysis

## INTRODUCTION

Samara is an important class of seeds in population establishment because they can be dispersed a long distance with wind. Samara usually has a couple of wings with seed wrapped inside. The green samara wing was reported to have photosynthetic activity (Ashton, 1989; Kenzo *et al*., 2003) and may provide a proportion of the carbon requirement for seed development (Asxhan & Pfanz, 2003). However, little information on photosynthesis of samara was available.

Siberian elm (*Ulmus pumila* L.), a deciduous tree species, is widely planted along roadsides in northwestern China. It possesses samara with a couple of flat and oval membranous wings. The seed was wrapped inside the wings. The samaras of Siberian elm appear in April, about one week before leaves emerge. In April, air temperature in northwestern China fluctuates drastically and often drops acutely to chilling or freezing temperatures. Therefore, samara may often experience chilling and freezing stresses.

Chilling or freezing stress has adverse impacts on antioxidant defense systems (Lee & Lee, 2000), photosynthesis (Cavender-Bares, 2007) and growth of plant (Inouye, 2000; Tsarouhas *et al*., 2000). The photosynthetic apparatus in leaf, especially photosystem II (PSII), is well known to be sensitive to low temperature stress (Strauss *et al*., 2006; Cavender-Bares, 2007; Han *et al*., 2009). PSII reaction centers (Fryer *et al*., 1995), energy trapping and transfer in PSII (Levasseur *et al*., 1990; Jiang *et al*., 2006) and electron transport (Van Heerden *et al*., 2004, 2007; Strauss *et al*., 2006) have been demonstrated to be target sites with low temperature injury. However, effect of chilling or freezing temperature on the physiology of samara, especially photosynthesis, is still unknown.

When illuminated with high intensity actinic light, dark-adapted oxygenic photosynthetic organisms show the polyphasic rise with the basic steps from the 'origin' (O) through two 'inflections' (J & I) to a 'peak' fluorescence level (P) (Strasser & Strasser, 1995). The polyphasic fast-phase fluorescence induction curve provides valuable information on the magnitude of stress effects on photosystem II (PSII) function. Strasser and Strasser (1995) established a procedure for quantitatively calculating several phenomenological and biophysical parameters on the basis of O-J-I-P curve, known as the JIP-test. The fast rise Chl *a* fluorescence and the JIP-test has been proved to be a useful tool for the in vivo investigation of PSII function under various environmental stresses (Appenroth *et al*., 2001; De

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Ronde *et al*., 2004; Christen *et al*., 2007; Strasser *et al*., 2007; Pan *et al*., 2008 & 2009).

The objective of the present study was to investigate responses of PSII activity of samara and leaf of Siberian elm to chilling and freezing stress and their recovery by using the polyphasic Chl *a* fluorescence rise (OJIP) test.

## MATERIALS AND METHODS

**Plant materials and treatments at low temperature:** Siberia elm (*Ulmus pumila*) shoot cuttings with dozens of three-week old samaras and leaves were grown in glassy containers containing tap water in a growth chamber with 12 h photoperiod and photosynthetic photon flux density (PPFD) of 200 μmol $m^{-2}$ $s^{-1}$ and temperature of 22-25°C. The chlorophyll fluorescence of samara and leaf were monitored every hour. The JIP-test parameters for samara and leaf on cuttings grown in the tap water are stable for at least two days. Therefore, tap water was used as the supporting medium for cuttings in all experiments. The cuttings without treatment with low temperature were used as the control. Low temperature (-15, -5 & 5°C) was applied to the samaras and leaves in the darkness. For each measurement, those samaras or young leaves from cuttings of lower canopy under the treatment of three low temperatures were randomly selected for chlorophyll fluorescence tests.

**Measurements of chlorophyll *a* fluorescence transients:** Samples (samara & leaf) were adapted in the dark for 3 min before measurement of chlorophyll fluorescence. The chlorophyll fluorescence transient was recorded up to 1s on a logarithmic time scale, with a data acquisition every 10 μs for the first 2 ms and every 1ms thereafter, by the handheld fluorometer (PSI, Brno, CZ). Each measured O-J-I-P induction curve was analyzed according to the JIP-test (Strasser & Strasser, 1995). The following data were directly obtained from the fast rise kinetic curves: $F_o$, the initial fluorescence, was measured at 20 μs, at this time all reaction centers (RCs) are open; $F_J$ and $F_I$ are the fluorescence intensity at J step (at 2 ms) and I step (at 30 ms); Fm, the maximal fluorescence, was the peak fluorescence at P step when all RCs were closed after illumination; $F_{300\mu s}$ was the fluorescence at 300 μs. Selected JIP-test parameters quantifying PSII behavior were calculated from the above original data as the formulae in Table I (Strasser *et al*., 2004).

**Stastistical analysis:** All measurements were repeated at least three times. The statistical significance of the difference between two values was evaluated using Student's *t*-test at $p$=0.05. Post Hoc Tests used Student-Newman-Keuls test (S-N-K test) at $p$=0.05 level.

## RESULTS

**Difference in PSII activities between samara and leaf:** Typical O-J-I-P fluorescence transient curves of leaves and samaras of *U. pumila* were presented in Fig. 1. Fluorescence intensity from O-step ($F_0$) to P-step (Fm) for samaras was higher than those in leaves. Fluorescence for samara rise more rapidly from the minimum ($F_0$) to the maximum (Fm) than that for leaf, resulting in higher Fv value (= Fm - $F_0$) for samara than that for the leaf.

Significant difference in JIP-test parameters between samaras and leaves was observed (Table II). No significant difference in the maximum primary photochemical yield (Fv/Fm) between samara and leaf was found whereas the value of $PI_{ABS}$ for Samara was only 43.9% of that for leaf. Fv and $M_0$ for samara were 1.99 and 1.35 times for leaf, respectively. On the contrary, $S_m$ in the former was smaller than in the latter, about only 64 %.The electron transport rate and flux ($\varphi_{Eo}$ & $\Psi_o$) for samara were lower than those for leaf. On the contrary, the values of ABS/RC, $TR_o$/RC and $DI_o$/RC for samara were 48%, 48% and 46.2% higher than those for leaf.

**Effect of low temperature on PSII function:** Under chilling stress, the photosynthetic efficiency (Fv/Fm & $PI_{ABS}$) and electron transport activity ($\psi_o$, $\varphi_{Eo}$ & $ET_o$/RC) for leaf decreased significantly with treatment time while energy flux per RC (ABS/RC, $TR_o$/RC & $DI_o$/RC) increased with treatment time. However, for samara, the JIP-test parameters except $PI_{ABS}$ changed slightly after one-hour exposure to chilling temperature. Similarly, after one-hour chilling treatment, electron transport ($\psi_o$, $\varphi_{Eo}$ & $ET_o$/RC) was significantly inhibited for leaf but changed little for samara. The indexes of electron transport for both leaf and samara decreased progressively with exposure time. A 12-h dark chilling treatment led to decreases in $\psi_o$, $\varphi_{Eo}$ and $ET_o$/RC by 48.1%, 38.9% and 34.5% for leaf and 14.6%, 19.1% and 12.8% for samara, respectively. ABS/RC and $TR_o$/RC for leaf increased due to one-hour chilling stress and decreased to some extent but still above the values for the control. For samara under chilling stress, ABS/RC and $TR_o$/RC increased continuously with exposure time (Fig. 2).

For freezing stress experiments, effect of two freezing temperature (-5°C & -15°C) on PSII function were investigated. One hour of freezing at -15°C stress resulted in irrecoverable decreases in Fv/Fm and $PI_{ABS}$ for leaf by 82.9% and 100%, respectively. Similarly, most of the Fv/Fm and $PI_{ABS}$ for samara irrecoverably decreased after being exposed to -15°C for one hour. Therefore, extended freezing stress at -15°C had no further effect (Fig. 2).

Fv/Fm and $PI_{ABS}$ for both leaf and samara were reduced remarkably at -5°C. Fv/Fm and $PI_{ABS}$ decreased from 82.2% and 9.2% of the control to 75.9% and 5.9% of the control with exposure time increasing from 1 h to 12 h. For samara, Fv/Fm and $PI_{ABS}$ dropped to 92.5% and 43.2% after one-hour exposure to -5°C and changed little with prolonged stress. Electron transport ($\psi_o$, $\varphi_{Eo}$ & $ET_o$/RC) for both leaf and samara were substantially inhibited during the first hour at -5°C stress. Electron transport activity responded differentially for leaf and samara with exposure time being prolonged from 1 to 12 h. The values of $\psi_o$, $\varphi_{Eo}$

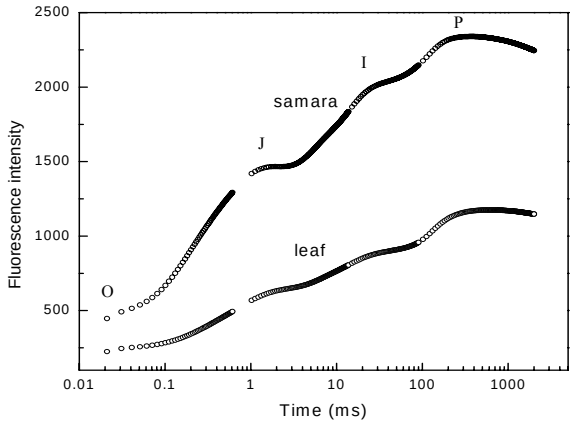
**Table I: Formulae and terms used in the JIP-test analysis**

| Term and formula | Definition |
|---|---|
| $F_o = F_{20\ \mu s}$ | minimal fluorescence, when all PSII RCs are open (at $t = 0$) |
| Fm = Fp | maximal fluorescence, when all PSII RCs are closed |
| $V_J \equiv (F_J - F_o)/(Fm - F_o)$ | relative variable fluorescence at the J-step |
| $M_o \equiv 4\ (F_{300\mu s} - F_o)/(Fm - F_o)$ | approximated initial slope [$ms^{-1}$] of the fluorescence transient |
| **Yields or flux ratios** | |
| $\varphi_{Po} \equiv TR_o/ABS = [1 - (F_o/Fm)] = F_v/F_M$ | maximum quantum yield of primary photochemistry (at $t = 0$) |
| $\Psi_o \equiv ET_o/TR_o = (1 - V_J)$ | probability (at $t = 0$) that a trapped exciton moves an electron into the electron transport chain beyond $Q_A$ |
| $\varphi_{Eo} \equiv ET_o/ABS = [1 - (F_o/Fm)]\ \psi_o$ | quantum yield of electron transport (at $t = 0$) |
| **Specific energy fluxes** | |
| $ABS/RC = M_o\ (1/V_J)\ (1/\varphi_{Po})$ | absorption flux per RC |
| $TR_o/RC = M_o\ (1/V_J)$ | trapped energy flux per RC (at $t = 0$) |
| $ET_o/RC = M_o\ (1/V_J)\ \Psi_o$ | electron transport flux per RC (at $t = 0$) |
| **Performance index at $t = 0$** | |
| $PI_{ABS} \equiv (RC/ABS)\ [\varphi_{Po}/(1- \varphi_{Po})][\psi_o/(1 - \psi_o)]$ | performance index on absorption basis |

**Table II: $M_o$, $S_m$, Fv/Fm, ABS/RC, $TR_o$/RC, $ET_o$/RC and $PI_{ABS}$ for leaves and samaras of *U. pumila*. The values were indicated mean ± S.E. ($n = 6$)**

| Type | Fv/Fm | $PI_{ABS}$ | $\varphi_{Eo}$ | $\Psi_0$ | ETo/RC | Mo | $S_m$ | ABS/RC | TRo/RC | $DI_o$/RC |
|---|---|---|---|---|---|---|---|---|---|---|
| Leaf | 0.81±0.00 | 2.80±1.14 | 0.46±0.01 | 0.57±0.01 | 0.93±0.02 | 0.7±0.02 | 665±4.8 | 2.01±0.04 | 1.62±0.04 | 0.38±0.01 |
| samara | 0.81±0.00 | 1.23±0.07 | 0.37±0.01 | 0.46±0.01 | 1.11±0.01 | 1.28±0.02 | 426±7.1 | 2.98±0.04 | 2.41±0.02 | 0.57±0.02 |

**Fig. 1: Chlorophyll *a* fluorescence transient (OJIP) of dark-adapted samaras and leaves in *U. pumila* plotted on a logarithmic time scale (20 µs -1 s)**




and $ET_o$/RC for leaf further decreased when stress is prolonged but remained at higher levels for samara (Fig. 2).

**Recovery potential from chilling or freezing stresses:** The recovery potential of maximum primary PSII photochemical yield (Fv/Fm) for leaf and samara after cessation of chilling or freezing stress was shown in Fig. 3. Fv/Fm recovered to 95.7% for 1-h chilled (5°C) leaf and 89.7% for 12-h chilled (5°C) leaf, respectively after 2-h recovery at room temperature; further exposure to this temperature had no effect. For samara, Fv/Fm continued to decline slightly after cessation of chilling stress. Unlike the chilling stress, Fv/Fm for both leaf and samara after exposed to freezing temperature (-5°C) recovered with the leaf having higher recovery rate than samara as the damage in leaf was more severe and had more room for recovery.

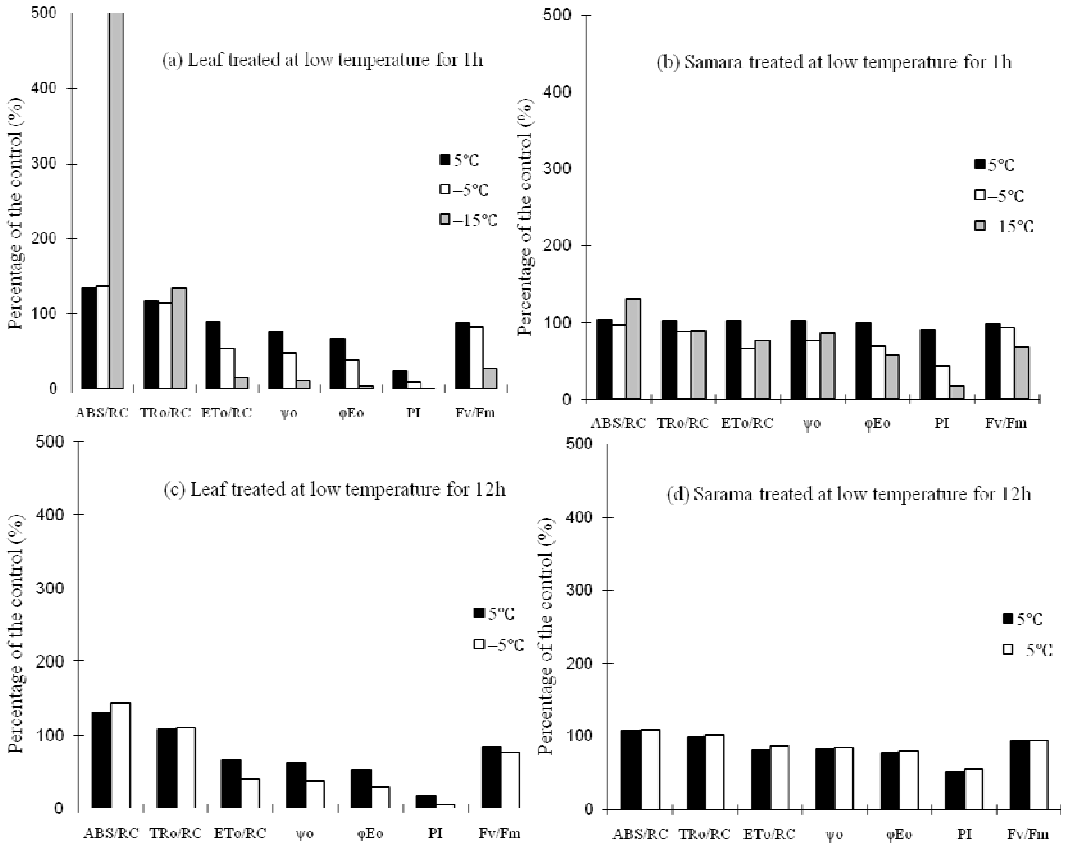
## DISCUSSION

The OJIP curve for samara lies above that for leaf i.e., higher fluorescence intensity at O, J, I and P steps (Fig. 1), indicating the difference in PSII activity between the samara and the leaf. The higher Fv for samara than leaf means that samara has higher PSII capacity to reduce plastoquinone (Bukhov *et al.,* 1987). Little difference in Fv/Fm between samara and leaf and the higher values of ABS/RC, $TR_o$/RC and $DI_o$/RC accompanied by lower values of $PI_{ABS}$, $\varphi_{Eo}$ and $\Psi$o for samara indicate that lower PSII performance of samara results from the difference of energy flux (ABS/RC) and electron transport ($\psi_o$) (Table II). The higher initial slope $M_o$ for samara reflects higher net rate of the RCs' closure and higher rate of $Q_A$ reduction at single turnover (Strasser *et al*., 2004). Relative low Sm for samara shows the smaller PQ pool at acceptor side for samara than that for leaf, which limits the reduction of $Q_A$ to $Q_A^-$ i.e., reopening of closed RC's.

Extensive previous studies showed that PSII function was inhibited under dark chilling or freezing stress (Janda *et al*., 1996; Strauss *et al*., 2006; Lin *et al*., 2007; Pagter *et al*., 2008). In the present study, JIP-test analysis clearly demonstrated that chilling temperature and freezing temperature had adverse impact on PSII function for both samara and leaf.

Decreases of electron transport activity ($\psi_o$, $\varphi_{Eo}$ & $ET_o$/RC) for both leaf and samara were induced by dark chilling (5°C) and freezing (-5°C & -15°C). Smaller deceases in electron transport activity ($\psi_o$, $\varphi_{Eo}$ & ETo/RC) in samara than leaf indicates that PSII of samara is less sensitive to low-temperature stress than leaf. The decrease of electron transport flux per RC ($ET_o$/RC) might result from the inhibition of electron transport in thylakoid induced by low-temperature stress (Klosson & Krause,

**Fig. 2: Effect of low temperature treatment on JIP-test parameters for leaf and samara of *U. pumila*: (a) JIP-test parameters for leaf after exposure to chilling (5°C) and freezing (-5°C and -15°C) stress for 1 h, (b) JIP-test parameters for samara after exposure to chilling (5°C) and freezing (-5°C and -15°C) stress for 1 h, (c) JIP-test parameters for leaf after exposure to chilling (5°C) and freezing (-5°C) stress for 12 h, and (d) JIP-test parameters for samara after exposure to chilling (5°C) and freezing (-5°C) stress for 12 h. These parameters are expressed as percentage of the control. Each data point represents the mean of three measurements**

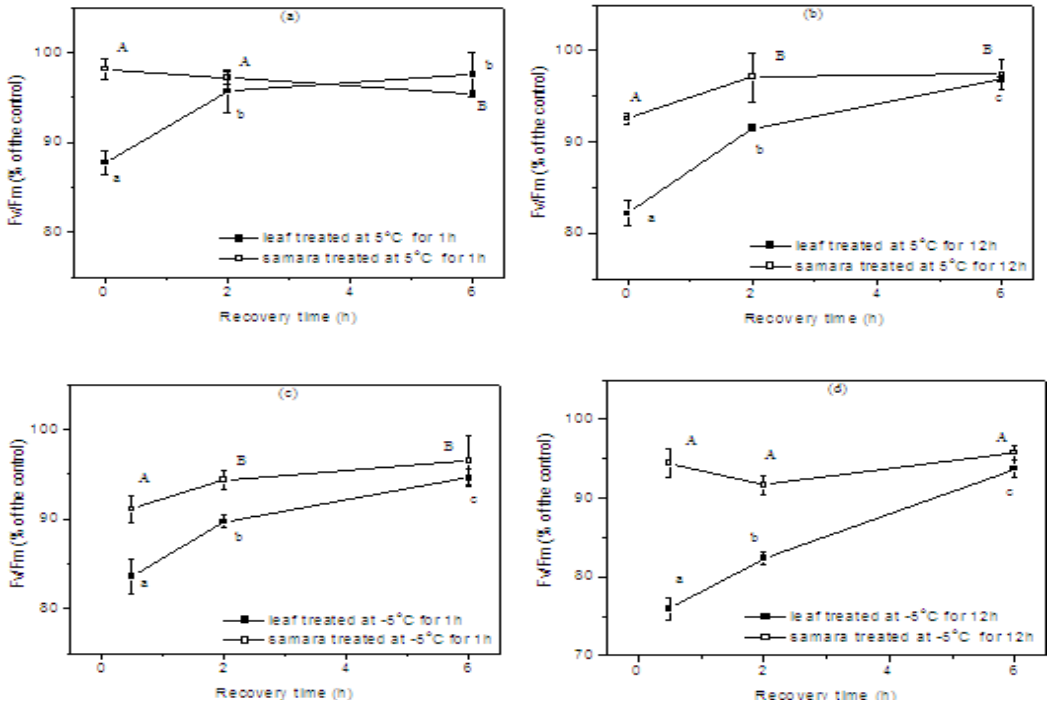



1981; Grafflage & Krause, 1986). The decrease of electron transport activity induced by low temperature can lead to energy imbalance between photochemistry and photosynthetic metabolism (Huner *et al*., 1998). Energy flux per RC (ABS/RC, TRo/RC & $DI_o$/RC) pronouncedly increased with decreasing temperatures for both samara and leaf (Fig. 2). When the absorbed light energy exceeds the capacity of the RCs to use the trapped energy through photosynthesis, the excess light energy have to be dissipated as heat in order to avoid PSII damage (Rapacz *et al*., 2004; Garstka *et al*., 2007; Mai *et al*., 2009). The regulation of energy transfer by increasing dissipation was considered to be a strategy for plants to protect themselves against low temperature stress (Rapacz *et al*., 2004; Corcuera *et al*., 2005).

The maximum quantum yield of primary photochemistry (Fv/Fm) is proven to be a useful indicator for evaluating the effects of low temperature on plants (Appenroth *et al*., 2001; Hermans *et al*., 2003; Thach *et al*., 2007). In most cases, Fv/Fm was found to be reduced under low temperature stress (Strauss *et al*., 2006; Pagter *et al*., 2008). In the present study, Fv/Fm for both samara and leaf of *U. pumila* decreased due to chilling or freezing. $PI_{ABS}$ showed similar change pattern to Fv/Fm in response to low temperature stress, indicating that vitality of PSII was suppressed. In a few previous studies, $PI_{ABS}$ were found to be a more sensitive parameter for characterizing the effect of low temperature on PSII activity than Fv/Fm (Rapacz, 2007; Rapacz & Woźniczka, 2009).

Fv/Fm has been used as an indicator for assessing recovery potential of PSII function from chilling and freezing stress (Corcuera *et al*., 2005). In the present study, Fv/Fm for samara or leaf after chilled at 5°C and frozen at -5°C shows a recovery trend. However, drastic and irreversible injuries occurred in samara and leaf after freezing at -15°C for 1 h. Rapacz (2007) reported that Fv/Fm for winter wheat frozen at -15°C for 7 d dropped to zero and no recovery was observed. Oak species from high latitude showed no decline in Fv/Fm under freezing stress at -10°C, whereas those species without acclimation

**Fig. 3: Recovery of Fv/Fm for leaf and samara after 5℃ chilling treatment for 1h (a), 12h (b) and after -5℃ freezing treatment for 1h (c), 12h (d). All values represent the average of three replicates. Data were shown as percentages of the control. The bars indicate standard error. Letters showed the significant differences**




underwent irreversible injury at the same condition (Cavender-Bares, 2007).

The samara of *U. pumila* was demonstrated to have photosynthetic activity. Photosynthesis in samara provides nutrient and energy for seed development before leaf expansion. Samara was more tolerant to freezing stress than leaf. For samara, the tolerance to freezing temperature and subsequent recovery capacity is important for avoiding freezing injury to its photosynthetic apparatus and maintaining continuous supply of nutrient and energy for its seed development. Therefore, it is of great ecological importance for population establishment of Siberian elm and its northern distribution limit in northern hemisphere.

In conclusion, exposure to chilling and freezing temperature led to harmful effects on PSII function in both samara and leaf of *U. pumila*. Low temperature stress decreased photochemical efficiency (Fv/Fm & $PI_{ABS}$) and electron transport activity ($\psi_o$, $\varphi_{Eo}$ & $ET_o$/RC) in both leaf and samara. Samara PSII showed higher tolerance to low temperature stress than leaf PSII. Photochemistry (Fv/Fm) for chilled (at 5°C) or frozen (at -5°C) samara or leaf could be recovered. Freezing at -15°C caused irreversible injuries to PSII in samara and leaf. The higher tolerance of samara PSII activity is important for seed development, population establishment and even the north distribution limit of Siberian elm in northern hemisphere.

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