

CO₂-EXCHANGE AND CHLOROPHYLL FLUORESCENCE RESPONSES OF FORAGE GRASSES DURING SALT STRESS AND RECOVERY IN QINGHAI-TIBETAN PLATEAU (QTP)

MUHAMMAD ARFAN^{1,4}♠, CHAO ZHANG²♠, DA-WEI ZHANG¹, DA-XU LI³, JIA-JUN YAN³, MING-HONG YOU³, SHI-QIE BAI³ AND HONG-HUI LIN^{1*}

¹Ministry of Education Key Laboratory for Bio-Resource and Eco-Environment, College of Life Science, State Key Laboratory of Hydraulics and Mountain River Engineering, Sichuan University, Chengdu, Sichuan 610064, China

²Liupanshui Normal College, Liupanshui, Guizhou 553000, China

³Sichuan Academy of Grassland Science, Chengdu, Sichuan 611731, China

⁴Department of Forestry, Range & Wildlife Management, The Islamia University of Bahawalpur 63100, Pakistan.

*Corresponding author's email: hmlin@scu.edu.cn

♠Authors contributed equally to this work

Abstract

Grassland degradation has become a core issue in research and policy due to recognition of the magnitude of anthropogenic and environmental threats in Qinghai-Tibetan Plateau (QTP). The problem of soil salinity could worsen in recent scenario of global change. Therefore, an urgent need exists to select salt efficient forage species for sustaining ecosystem services in changing environment. The aim of this study was to investigate different level of salinity treatments and, recovery situations in three forage species (*Avena sativa* L., *Elymus sibiricus* L., and *Phalaris arundinacea* L.). The results showed that increase in salinity level was associated with more symptoms of damage while recovery benefited with the effects being different among three species. Gas exchange (P_n rates and $iWUE$) and chlorophyll fluorescence (qP, NPQ, Fv/Fm and $\Phi PSII$) parameters revealed that performance of *Elymus sibiricus* was better during progressive salt stress followed by *Phalaris arundinacea*. However, *Phalaris arundinacea* performed better than *Elymus sibiricus* during recovery situation. Similar trend was observed through relative water content (RWC) and electrolyte leakage (EL) results. Taken together, these results demonstrated that *Elymus sibiricus* was relatively more salt-tolerant species, while *Phalaris arundinacea* exhibited better ability to recover in QTP environment. On the other hand, *Avena sativa* was screened as the most salt sensitive among these three species.

Key words: Qinghai-Tibetan Plateau (QTP); Salt stress tolerance; Gas exchange; Chlorophyll fluorescence; Relative water content, Electrolyte leakage.

Abbreviations: FV/FM, the maximum photochemical efficiency of PSII; NPQ, non-photochemical quenching coefficient; $iWUE$, instantaneous water use efficiency; PN, net photosynthetic rate; qP, photochemical quenching; $\Phi PSII$, actual photochemical efficiency.

Introduction

The alpine grassland is mainly found on Qinghai-Tibetan Plateau (QTP) in western part of China (Sun, 2005). This region has become crucial for researchers as it represent the most intensive distribution of world's alpine ecosystem (Brandt *et al.*, 2013; Chen *et al.*, 2014). In a previous study by He *et al.*, (2008) it was revealed that alpine grassland productivity in QTP declined significantly over the recent half-century. The grassland degradation has become an important issue in this region because of climate change and anthropogenic effects (Zhang *et al.*, 2007; Yang *et al.*, 2013; Qin *et al.*, 2014). In recent years, the restoration of degraded alpine grassland has become a challenge (Ma *et al.*, 2007; Zhao, 2011) as it directly associates with local economy (Liu *et al.*, 1999).

There is an urgent need to identify key factors which could possibly involve in restoring particular area. Degradation could negatively affect soil functionality by changing soil acidification and salinization level (Krasilnikov *et al.*, 2016). Degradation of soil through salts has threatened forage and cattle production in arid and semi-arid regions around the world (Bossio *et al.*, 2007). Semi-arid rangeland degradation in western China demonstrates highest salinization degree affecting 2.3% of total country area. One effective method to sustain

ecosystem services could be to introduce salt efficient species exhibiting excellent qualities to survive in changing environment (Qin *et al.*, 2010). Salinity can cause many adverse effects including low osmotic potential, specific ion toxicity and nutrients imbalance (Läuchli & Epstein, 1990). These irregularities affect plants growth and development especially photosynthetic and leaf relative growth rate (Munns, 2005). However, the mechanisms of salinity tolerance in plants are not straightforward. To improve salt tolerances plants require many physiological adaptations to environment. In plants, photosynthesis process is primarily affected by salinity (Chaves, 1991; Munns *et al.*, 2006). Several previous studies described salt-tolerant and salt-sensitive species response to salinity stress (Percy & Ustin, 1984; Seemann & Critchley, 1985). Despite causing damaging effects to stomatal features, salinity can also affect PSII. Chlorophyll fluorescence is considered as an effective method used to screen varieties for salinity tolerance (Maxwell & Johnson, 2000; Naseer *et al.*, 2017). In a study, Abdeshahian *et al.*, (2010) reported that PSII was more prone to damaging effects of salinity by increasing NPQ, decrease in qP, Fv/Fm and $\Phi PSII$ in wheat plants. Garg & Garg (1982) in a previous study stated that increased salinity level could decrease chlorophyll content. Soil salinity could increase in recent scenario of global change. Therefore, we need to focus on identifying salt

tolerant species which could sustain ecosystem services (Yarsi *et al.*, 2017). The role of salt stress in inducing oxidative damage has been investigated previously (Stepien & Klobus, 2005). However, the extent to which species can differ in response to environmental factors is not well studied.

The study was conducted in Qinghai-Tibetan Plateau, China. The perennial grass *Elymus sibiricus* is an important forage grass due to strong adaptability, high crude protein content, and good palatability. It played a major role in restoration of natural grassland in QTP (Lu, 1993; Yan *et al.*, 2007). On the other hand, *Phalaris arundinacea* (reed canary grass) is considered as an important potential bioenergy crop due to its higher apparent productivity and strong ability of asexual reproduction (Zhang *et al.*, 2013). Moreover, *Phalaris arundinacea* exhibited broad range due to drought and salinity stress tolerance (Ge *et al.*, 2012; Zhang *et al.*, 2013; Tanka *et al.*, 2013). Previously we studied photoprotective mechanisms in *Phalaris arundinacea* to alleviate photoinhibition of PSII in QTP (Zhang *et al.*, 2017). Further, *Avena sativa* is used as source of fiber for cattle, the oat hay is widely used in traditional alpine farming systems of the QTP (Long, 1995; Dong *et al.*, 2003; Wu, 2007). These all three species are economically important for this area and differ in quality and quantity of forage. However, their salt tolerance and recovery patterns have never been tested either in the field or under laboratory conditions. In order to understand the dynamic responses of these forage grasses to salinity, we conducted a manipulation experiment in 2015–2016 to investigate their efficiency in QTP, the forage grasses were exposed to three different possible scenarios: (1) increasing salt stress (2) recovery situation (3), and salt adaptability test. The main objective of this study was to select species that exhibits excellent salt-tolerance through exploring major physiological processes.

Materials and Methods

Plant material and salinity manipulation plots experiment: We conducted this study in a greenhouse located at the Sichuan Academy of Grassland Science. The Qinghai-Tibetan Plateau is characterized as alpine ecosystem in Sichuan province of China (Rehovot, 31.47°N, 102.33°E) at an altitude of 3504 m. The warmest air temperature is in July (24.1°C) and the

coldest is in January (−10.3°C), while the mean annual precipitation is around 650 mm (SMB, undated; Yao *et al.*, 2000). The mean minimum temperatures and average maximum temperatures were 5°C and 15°C, respectively during the growing season (April–September). However, because of high solar radiation level in this area, the maximum leaf temperature during day could be 3–10°C higher than air temperature. The manipulated salinity experiment was performed in 2015–2016 to examine the effects of salinity on 3 grassland species (*Avena sativa* L., *Elymus sibiricus* L., and *Phalaris arundinacea* L.). Seeds of these species were first germinated in the field, and the plots were 6 m × 6 m and the row spacing was 0.2 m in 2014. Then, the two years old tiller buds of these species were transplanted from the field to flowerpot. Following soil composition was maintained in flowerpot: 10% perlite, 10% vermiculite, 10% nutrition soil, 10% sand and 60% garden soil from Hongyuan research base (soil pH of 6.312, organic matter of 7.58%). The greenhouse was completely covered and plants only received water from irrigation throughout the experiment.

During the growing season (mid-April to mid-September), salinity treatments were applied in three different regimes to represent a realistic soil or groundwater salinity level that could occur in Hongyuan, China and covered the range from low to moderate NaCl concentrations (0–400 mM). The altered growing season salinity regime produces: (i) The primary plots were the four salinity treatments with different NaCl concentrations 0, 125, 250 and 400 mM 85 days after transplantation (DAT) which were termed as T1, T2, T3 and T4, respectively (Fig. 1a), (ii) rainfall event displayed recovery situation, where plants cultivated with different NaCl concentrations 125, 250 and 400 mM at 85–105 DAT were irrigated 0 mM deionized water 105 DAT. These were termed as T5 (125+0 mM), T6 (250+0 mM) and T7 (400+0 mM), respectively (Fig. 1b), and (iii) Salt adaptability test increased salinity event, where plants cultivated with different NaCl concentrations 0, 125 mM at 85–105 DAT, and then more NaCl added (400mM) in this experiment 105 days after transplantation. These were termed as T8 (0+400 mM), T9 (125+400 mM), respectively (Fig. 1c). Above mentioned, scenarios helped us to understand different species response tolerance at altered salinity regimes and also their recovery patterns.

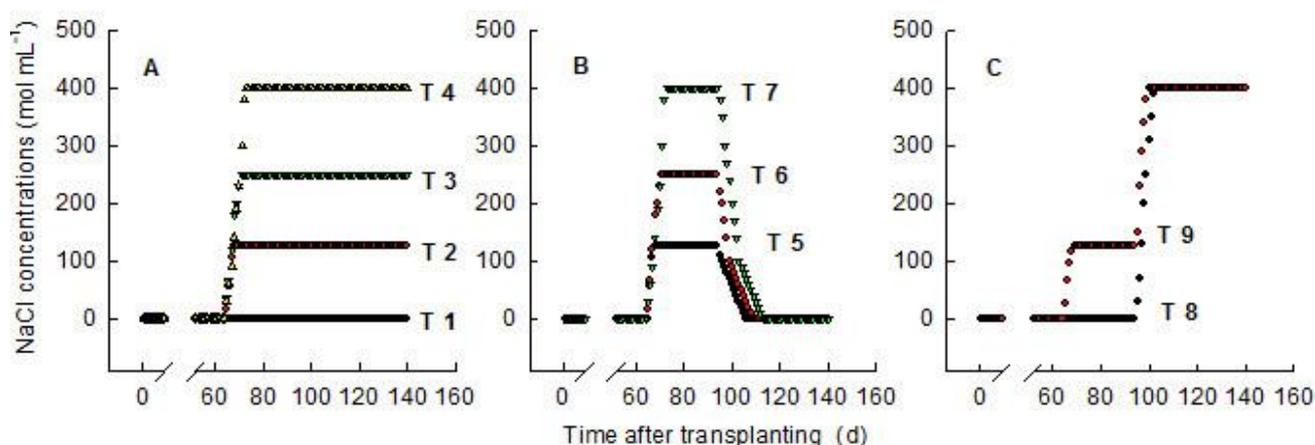


Fig. 1. Progressive salt stress test (A), desalination test (B), and salt adaptability test (C).

Gas exchange and chlorophyll fluorescence parameters measurements: Net photosynthesis, stomatal conductance, transpiration, efficiency of light harvesting of photosystem II and quenching coefficient were measured after 85, 95, 105, 125 and 135 DAT at different NaCl concentrations level. The measurements were performed in green house conditions and on leaves that were exposed to direct sun light. Gas exchange measurements were performed using the Li-6400 portable photosynthesis and fluorescence system (6400-40 leaf-chamber fluorometer; LiCor Inc.). All measurements for an experiment were completed in the same day between 10:00 and 15:00 h. For measurements of light response curve, leaves were first measured at 2,000 μmol (photon) $\text{m}^{-2} \text{s}^{-1}$ of photo-synthetically active radiation (PAR), and then the photo-synthetically active radiation was declined to 1,800, 1,000, 500, 200, 100, and 0 μmol (photon) $\text{m}^{-2} \text{s}^{-1}$. The chamber CO_2 concentration was kept at 400 $\mu\text{mol} \text{mol}^{-1}$ and leaf temperature was kept 15°C. For measurements of A-Ci response curves, leaves were first measured at 0 $\mu\text{mol} \text{mol}^{-1}$ of chamber CO_2 , and then the CO_2 concentration was increased stepwise up to 2,000 $\mu\text{mol} \text{mol}^{-1}$. Light intensity was kept at 2,000 μmol (photon) $\text{m}^{-2} \text{s}^{-1}$ and leaf temperature was kept 15°C. Also, the gas exchange parameter and Chlorophyll fluorescence parameter were monitored on leaves at 0 μmol (photon) $\text{m}^{-2} \text{s}^{-1}$ in 5:00–6:00 h. For recording data for different gas exchange attributes such as net CO_2 assimilation rate (P_n), intercellular CO_2 concentration (C_i), and transpiration rate (T_r) and stomatal conductance (G_s), a fully expanded second leaf of one plant from each replicate was used. Values for and were calculated using the equations of von Caemmerer & Farquhar (1981). Instantaneous water use efficiency ($i\text{WUE}$) was calculated as the ratio between net photosynthesis and transpiration (Condon *et al.*, 2002). Fluorescence parameters such as maximum photochemical efficiency were measured using (F_v/F_m) while actual photochemical efficiency, ΦPSII was calculated using the following equation ($F_m' - F_0$)/ F_m' of Genty *et al.*, (1989). Photochemical quenching (qP) was calculated as indicated by the manufacturer's manual for the LI-6400-40 leaf chamber fluorometer ($F_m' - F_s$)/($F_m' - F_0$), where F_s is the "steady-state" fluorescence. Whereas, non-photochemical quenching coefficient (NPQ) = ($F_m - F_m'$) / F_m' (Bilger *et al.*, 1990). Further, 125 DAT, the maximum photochemical efficiency (F_v/F_m) and the actual photochemical efficiency (ΦPSII) were determined by a chlorophyll fluorescence imager using an internal program (CF Imager; Technologica, UK).

Determination of leaf relative water content and electrolyte leakage: Relative water content (RWC) was determined as described previously by (Zhang *et al.*, 2014) and was calculated using the formula [(FW-DW)/(TW-DW)] \times 100, where FW corresponds to the fresh weight, TW the turgid weight after 24 h rehydrating of leaf samples at 4°C in the dark and DW the dry weight of leaf samples after 24 h at 80°C.

Plasma membrane damage was assessed using the method as described by Tang *et al.*, (2014). Approximately 0.3 g of fresh leaves were cut into 1 cm

pieces and placed into test tubes containing 30 mL of deionized water. Subsequently, initial electrical conductivity (EC_1) was measured. Samples were than boiled at 100°C for 30 min to achieve 100% electrical conductivity (EC_2). The relative conductivity of plasma membranes was calculated based on the ratio of electrical conductivity before and after boiling.

Statistical analysis

Means of three biological replicates were measured. Analysis of variance (ANOVA) was performed for comparison between different treatments. A difference was considered to be statistically significant when $p < 0.05$.

Results

Effect of salinity on gas-exchange parameters of 3 forage species: Difference among species in net photosynthesis P_n and $i\text{WUE}$ was found during all three scenarios (Fig. 1). A significant decrease in gas exchange parameters revealed that salinity in Qinghai-Tibetan Plateau negatively influenced all 3 species ($\text{PAR} = 2,000 \mu\text{mol} \text{m}^{-2} \text{s}^{-1}$). At the increasing salinity treatments (Fig. 1a) during 85-135 DAT, P_n rates and $i\text{WUE}$ efficiency was reduced 80% and 58% in *Phalaris arundinacea* whereas, in *Elymus sibiricus* these were decreased 83% and 42%, respectively. Further, after 105 days *Avena sativa* (T4) plant died due to reduction in both gas exchange parameters (Figs. 2 & 3a). On the other hand during recovery situation (Fig. 1b) between 105-125 DAT, plants performed better by increasing P_n rates (Fig. 2b) and $i\text{WUE}$ efficiency (Fig. 3b). Here, *Phalaris arundinacea* P_n rates and $i\text{WUE}$ efficiency after 105 and 125 days were significantly higher than other two species. While, *Avena sativa* T7 plant could not survive to adverse salinity after 125 days. Hence, *Phalaris arundinacea* recovery exhibited stable P_n rates (Fig. 2b) and $i\text{WUE}$ efficiency (Fig. 3b) followed by *Elymus sibiricus*. For salinization experiment (Fig. 1c), P_n rates (Fig. 2c) and $i\text{WUE}$ efficiency (Fig. 3c) significantly decreased more in *Phalaris arundinacea* compare to *Elymus sibiricus*. Whereas, *Avena sativa* plants could not survive in adverse salinity after 125 days (Fig. 2 & 3c). Gas exchange parameters screened *Elymus sibiricus* as the most stable species during salinity however, *Phalaris arundinacea* exhibited better recovery.

The influence of salinity on plants chlorophyll fluorescence and related parameters: The perturbation of leaf metabolism by salinity was detected with CF Imager, the quantum yield (F_v/F_m) was recorded after 30 min of dark adaptation. Actual quantum yield (ΦPSII) was measured on the fully expanded leaves that were illuminated for 30 min in the growth chamber with actinic light at a PPFD 1,000 $\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$ after dark adaptation. In this study, three different salinity situations were used to identify *Avena sativa*, *Elymus sibiricus*, and *Phalaris arundinacea* responses after 105 days of transplantation (Fig. 1). During progressive salt stress tolerance experiment

(Fig. 1a), values of Fv/Fm and Φ PSII were higher in *Elymus sibiricus* for (T1, T2, T3 and T4) followed by *Phalaris arundinacea* whereas, *Avena sativa* appeared least tolerant under salinity stress conditions (Fig. 4a, b). Here, *Elymus sibiricus* Fv/Fm values appeared red, and were easily distinguished better from the leaves of other plants. Similarly, Φ PSII values were also higher in *Elymus sibiricus* (from red to green) displaying pattern of tolerant species while remaining two species were more towards blue color demonstrating intolerance. Moreover, for recovery observation plants were irrigated with fresh water under different level of salinity (Fig. 1b). This recovery experiment treatments (T5, T6 and T7), results revealed improved performance by all species (Fig. 4a, b). Here, recovery situation was more obvious in *Phalaris arundinacea* followed by *Elymus sibiricus* from Fv/Fm values (towards red color). Also, from Φ PSII *Phalaris arundinacea* performed better (towards red from green and blue color) compared to *Elymus sibiricus* (exhibiting green color dominance). While, *Avena sativa* revealed poor recovery conditions through both Fv/Fm and Φ PSII parameters. Finally, plants were exposed to increase salinity level (Fig. 1c). In treatments (T8 and T9), the leaves of *Avena sativa* exhibited lower values of Fv/Fm and Φ PSII, which screened her relatively intolerant. Here, *Elymus sibiricus* exhibited higher values of fluorescence parameters followed by *Phalaris arundinacea* (Fig. 4a, b). These fluorescence parameters screened *Elymus sibiricus* as the most salt tolerant species. However, *Phalaris arundinacea* exhibited better ability to recover in QTP environment.

Further, two important chlorophyll fluorescence parameters photochemical quenching coefficient (qP) and non-photochemical quenching (NPQ) were observed to assess salinity tolerance. During progressive salinity treatments (Fig. 1; a), qP was decreased while NPQ was increased (Fig 5 & 6) ($\text{PAR} = 2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$). Between 85-135 DAT (T4), qP values increased 3% in *Elymus sibiricus* than *Phalaris arundinacea* (Fig. 5a) whereas for NPQ values were decreased 2% in *Phalaris arundinacea* compared to *Elymus sibiricus* (Fig. 6a). Slight difference was observed between these two species. However, *Avena sativa* (T4) plant died due to salt sensitive nature after 105 days (Fig. 5 & 6a). Additionally, recovery situation was tested to understand plants response (Fig. 1; b). Here, qP values after 125 days (T7) were found significantly higher in *Phalaris arundinacea* followed by *Elymus sibiricus* (Fig. 5b). Further, NPQ values were significantly declined during recovery experiment 125 DAT. Here, NPQ values (T7) significantly decreased more for *Phalaris arundinacea* followed by *Elymus sibiricus* (Fig. 6b). Noticeably, *Avena sativa* T7 plants could not recover after 125 days (Fig. 5 & 6b). These results indicated that *Phalaris arundinacea* species could efficiently recover under higher salinity conditions. In last, salinization experiment performed according to (Fig. 1c). qP was significantly increased in *Elymus sibiricus* than *Phalaris arundinacea* (Fig. 5c) Whereas, *Elymus sibiricus* NPQ

was significantly decreased compared to *Phalaris arundinacea* after 125 days (Fig. 6c). However, *Avena sativa* plants could not survive in adverse salinity after 125 days (Fig. 5 & 6c). Taken together, (Fv/Fm and Φ PSII) *Elymus sibiricus* was found as salt tolerant.

The difference of leaf relative water content and electrolyte leakage in 3 forage species under salt stress:

In first condition, plants were exposed to progressive salinity stress (Fig. 1a). Leaf RWC of all three species (*Avena sativa* L., *Elymus sibiricus* L., and *Phalaris arundinacea* L) was decreased by increasing salinity and duration, especially in T3 and T4 after 125 and 135 days. RWC under 400-mM (T4) treatment was considerably lower in *Avena sativa* which resulted death of plant (105 DAT). Further, in both *Phalaris arundinacea* and *Elymus sibiricus* RWC was decreased about 35% between 85 to 135 days (Fig. 7a). During recovery experiment (Fig. 1b), RWC was increased in treatments (T5, T6 and T7), for *Avena sativa* (12%, 38% and X), *Phalaris arundinacea* (12%, 20% and 24%) and *Elymus sibiricus* (8%, 6% and 8%) between 105 to 125 days (Fig. 7b). However, *Avena sativa* (T7) plants died due to severe stress conditions (125 DAT). These results exhibited better recovery in *Phalaris arundinacea*. In last, adaptability test (Fig. 1c), *Avena sativa* plants of both T8 and T9 died 125 DAT. However, RWC in *Phalaris arundinacea* was decreased for T8 and T9 (52% and 40%) whereas, *Elymus sibiricus* exhibited (36% and 30%) decline for RWC in T8 and T9 between 105 to 125 days (Fig. 7c). Overall, these results demonstrated *Elymus sibiricus* as the most stable species under salinity whereas *Phalaris arundinacea* showed better recovery.

Further, electrolyte leakage (EL) was investigated from leaves of all three species. Increasing salinity was associated with increase in EL while recovery benefited all three species. First, plants were exposed to increasing salt stress (Fig. 1a). Leaf EL of all three species (*Avena sativa* L., *Elymus sibiricus* L., and *Phalaris arundinacea* L) increased rapidly with increasing salinity and period, especially in T3 and T4 during 125 and 135 days. Noticeably, EL under 400-mM (T4) treatment was significantly higher in *Avena sativa* causing death of plant (after 105 days), while both in *Phalaris arundinacea* and *Elymus sibiricus*, EL was increased approximately 58% between 85-135 days (Fig. 8a). During recovery experiment (Fig. 1b), EL was decreased in treatments (T5, T6 and T7), for *Avena sativa* (17%, 37% and X), *Phalaris arundinacea* (23%, 38% and 48%) and *Elymus sibiricus* (0%, 13% and 24%) between 105 to 125 days (Fig. 8b). *Avena sativa* (T7) could not recover after 125 days and died. In last, adaptability test (Fig. 1c), *Avena sativa* plants of both T8 and T9 died after 125 days. However, *Phalaris arundinacea* showed increased EL for T8 and T9 (90% and 60%), whereas *Elymus sibiricus* exhibited least EL by 64% in both T8 and T9 after 125 days (Fig. 8c). Therefore, *Elymus sibiricus* maintained EL better compared to other species under salt stress.

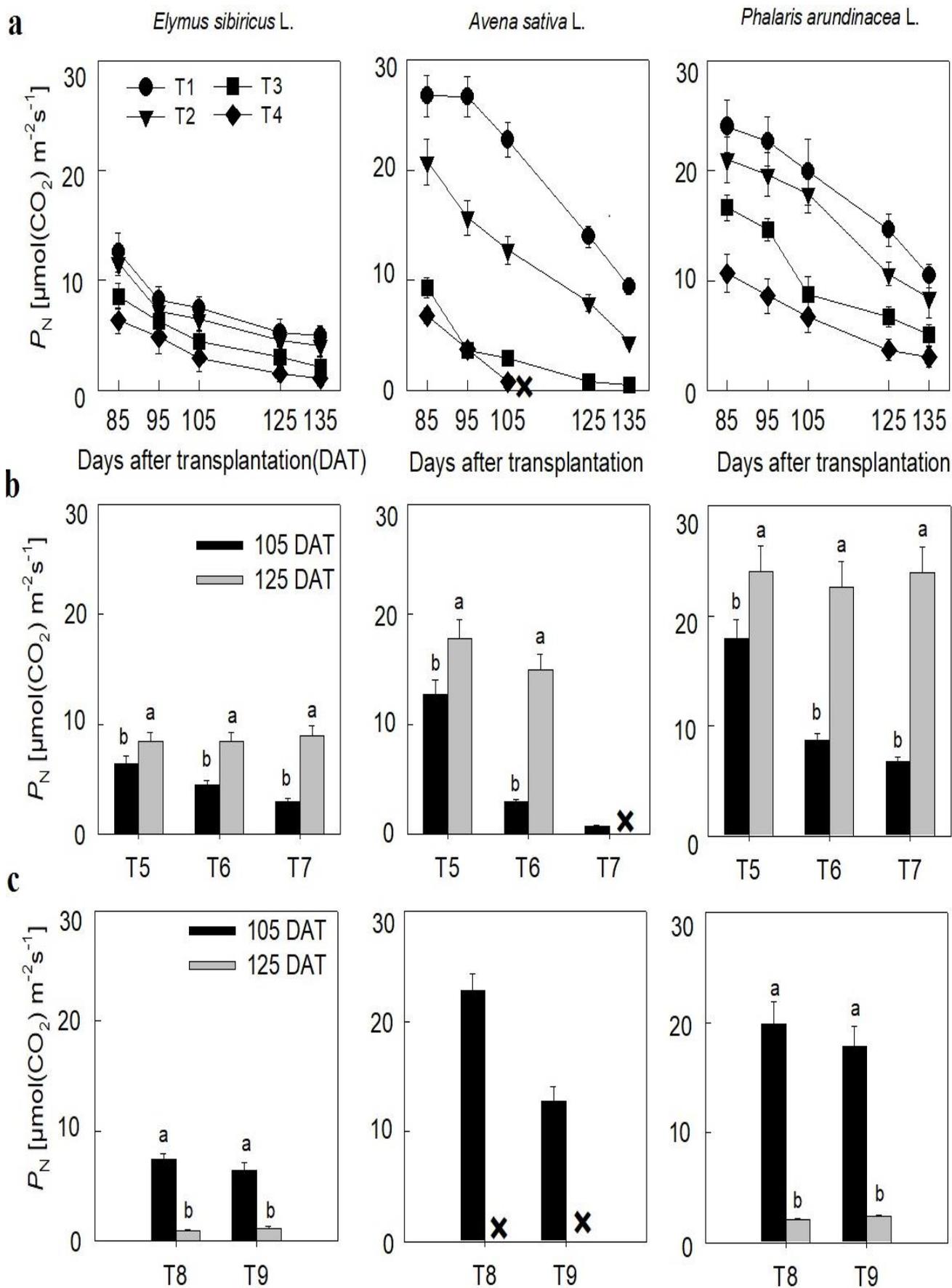


Fig. 2. Photosynthetic rates (P_N) in 3 grassland species (*Avena sativa* L., *Elymus sibiricus* L., and *Phalaris arundinacea* L.). Here, 3 grassland species were grown at different salt concentrations (Fig. 1), where X was the plant's death. These were measured at five different developmental stages (PAR = 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Mean \pm SE; n = 5).

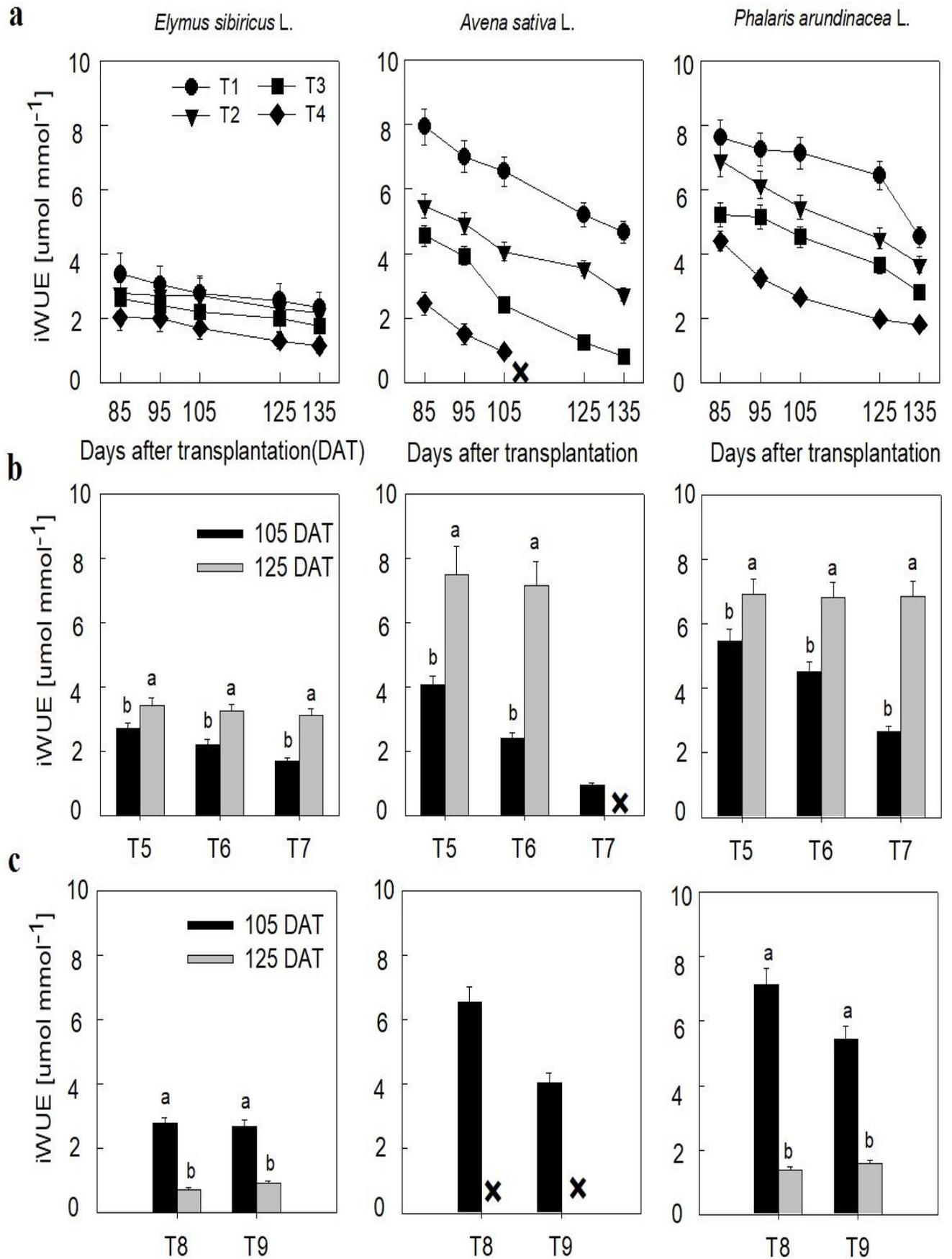


Fig. 3. Instantaneous water use efficiency (*iWUE*) in 3 grassland species (*Avena sativa* L., *Elymus sibiricus* L., and *Phalaris arundinacea* L.). Plants were grown at different salt concentrations (Fig. 1), where X was the plant's death. These were measured at five different developmental stages (PAR = 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Mean \pm SE; n = 5).

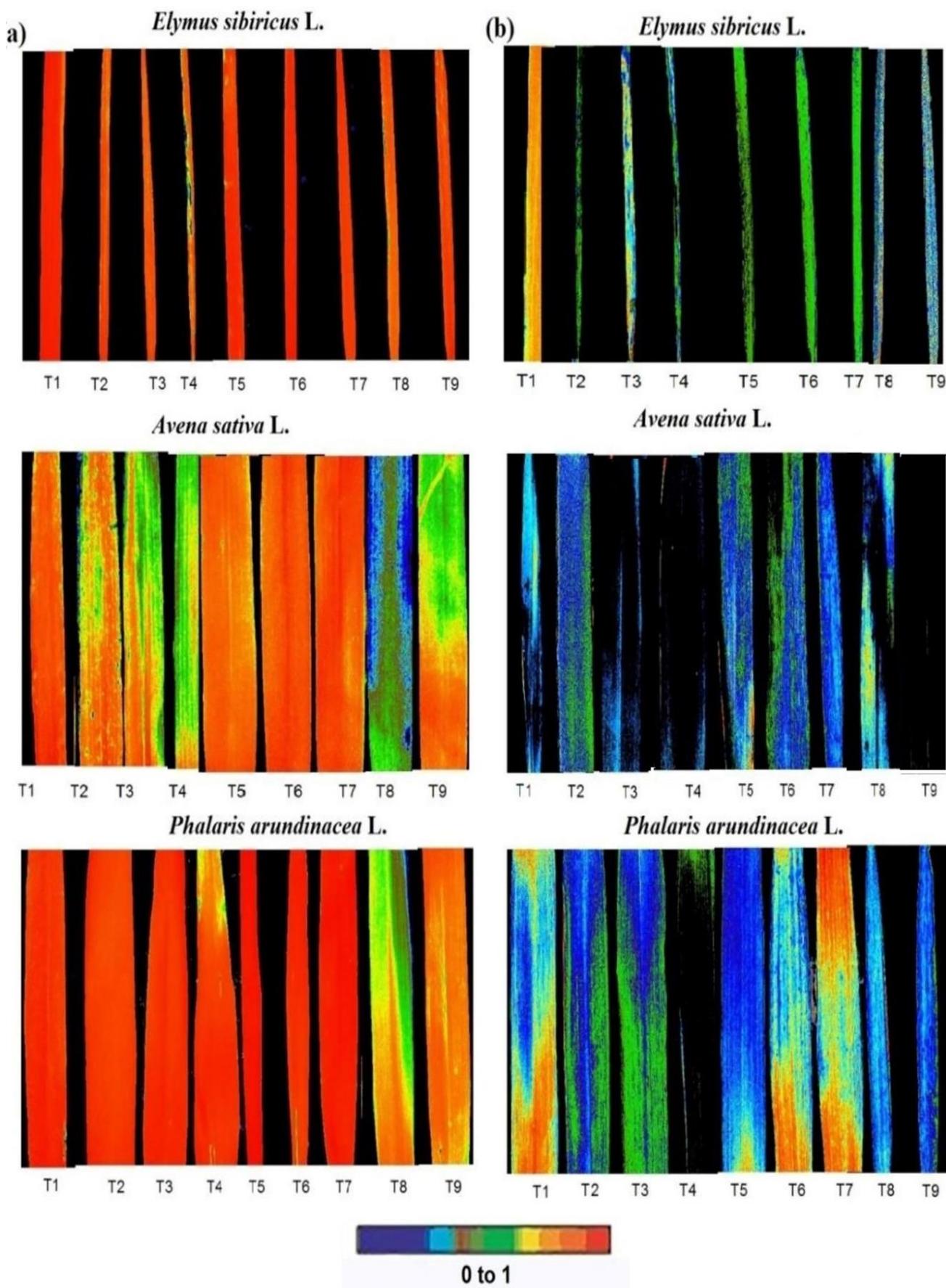


Fig. 4. Detection of maximum photochemical efficiency (Fv/Fm) (a) and actual photochemical efficiency (Φ PSII) (b) in 3 grassland species (*Avena sativa* L., *Elymus sibiricus* L. and *Phalaris arundinacea* L.). Plants were grown at different salt concentrations (Fig. 1). For each image, color bar represents the range of values from 0 to 1. These were measured at five different developmental stages.

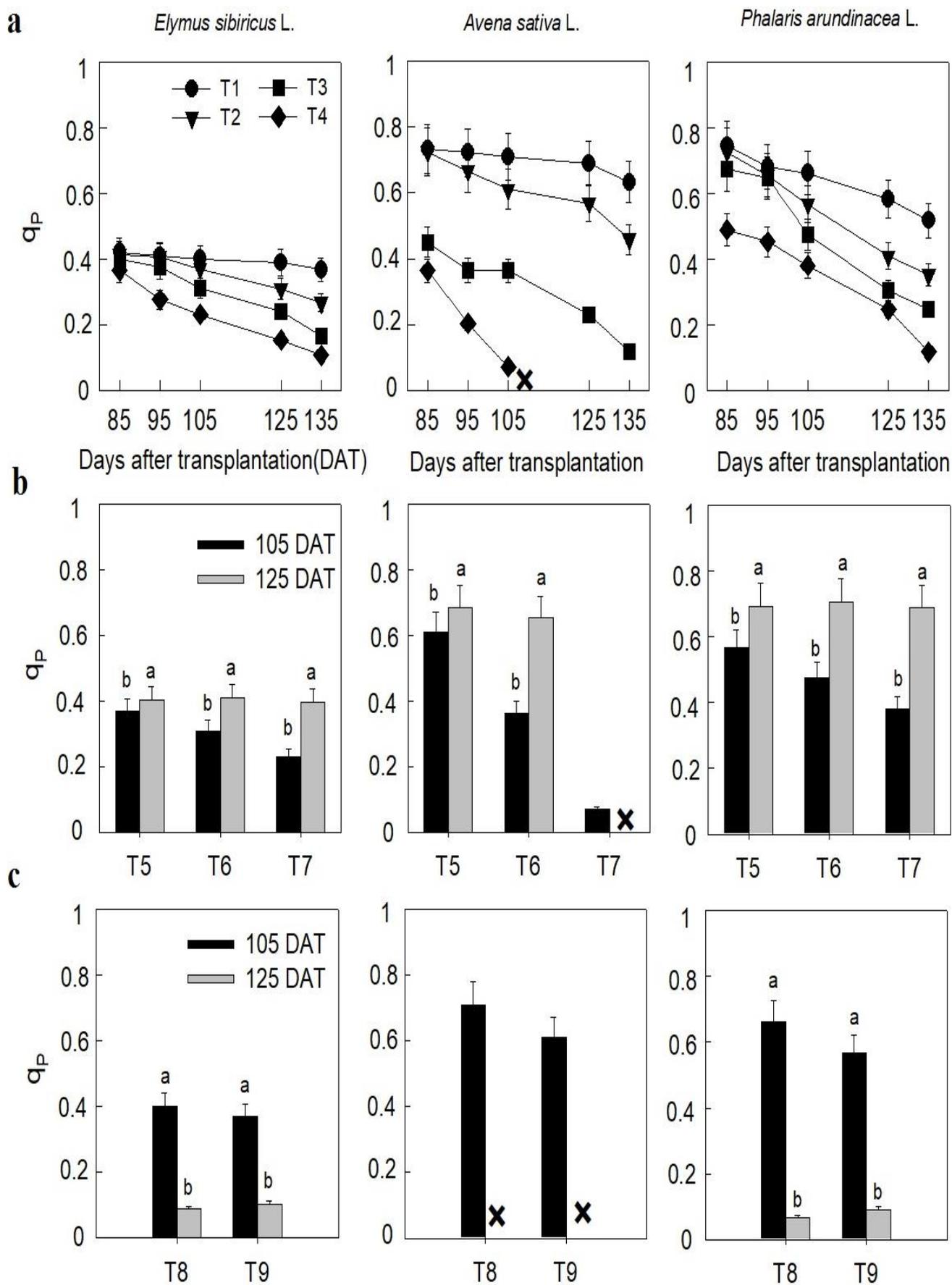


Fig. 5. The photochemical quenching coefficient (qP) in 3 grassland species (*Avena sativa* L., *Elymus sibiricus* L., and *Phalaris arundinacea* L.). Plants were grown at different salt concentrations, where X was the plant's death. These were measured at five different developmental stages (PAR = 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Mean \pm SE; n = 5).

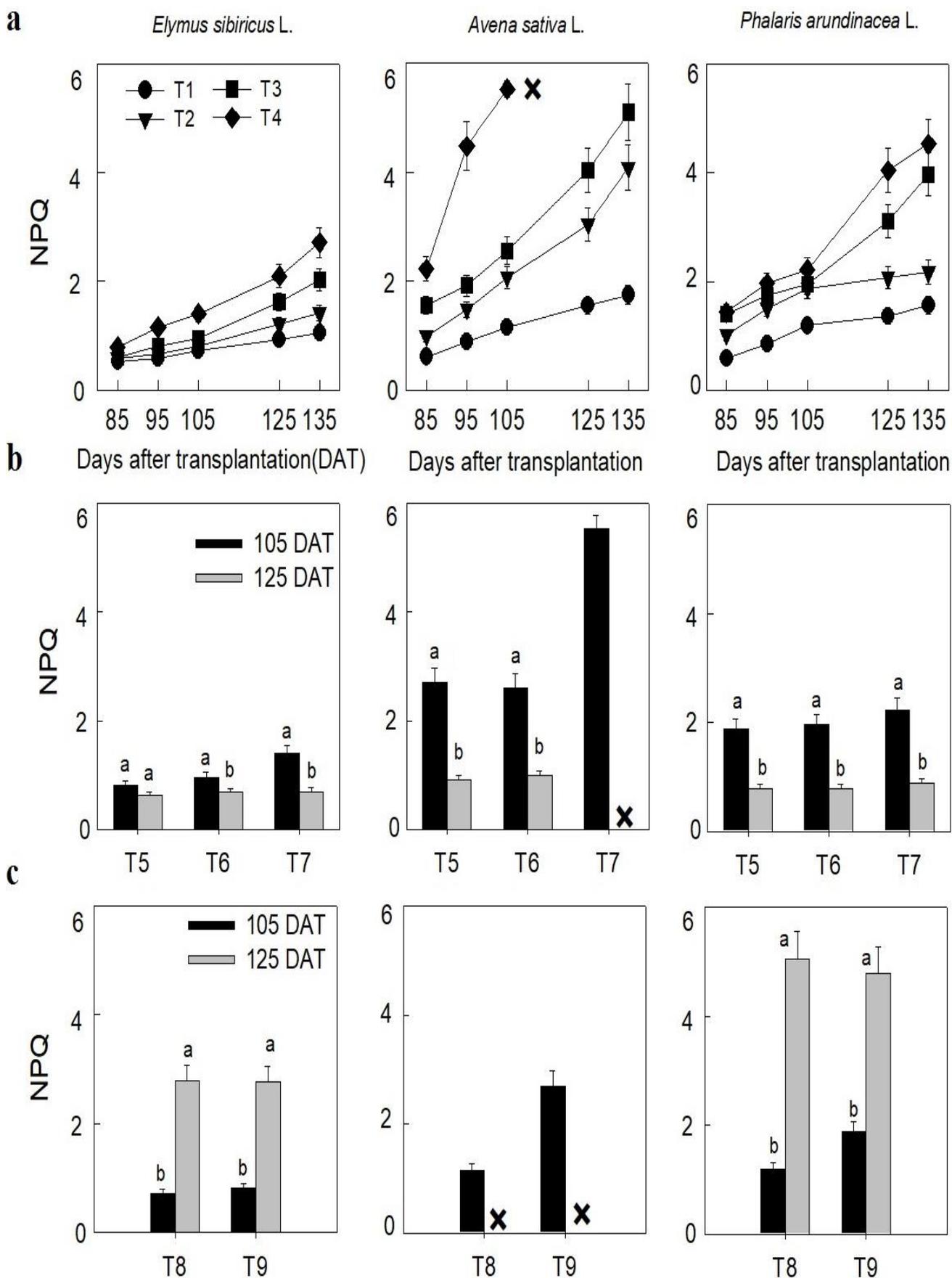


Fig. 6. The non-photochemical quenching coefficient (NPQ) in 3 grassland species (*Avena sativa* L., *Elymus sibiricus* L., and *Phalaris arundinacea* L.). Plants were grown at different salt concentrations, where X was the plant's death. These were measured at five different developmental stages (PAR = 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Mean \pm SE; n = 5).

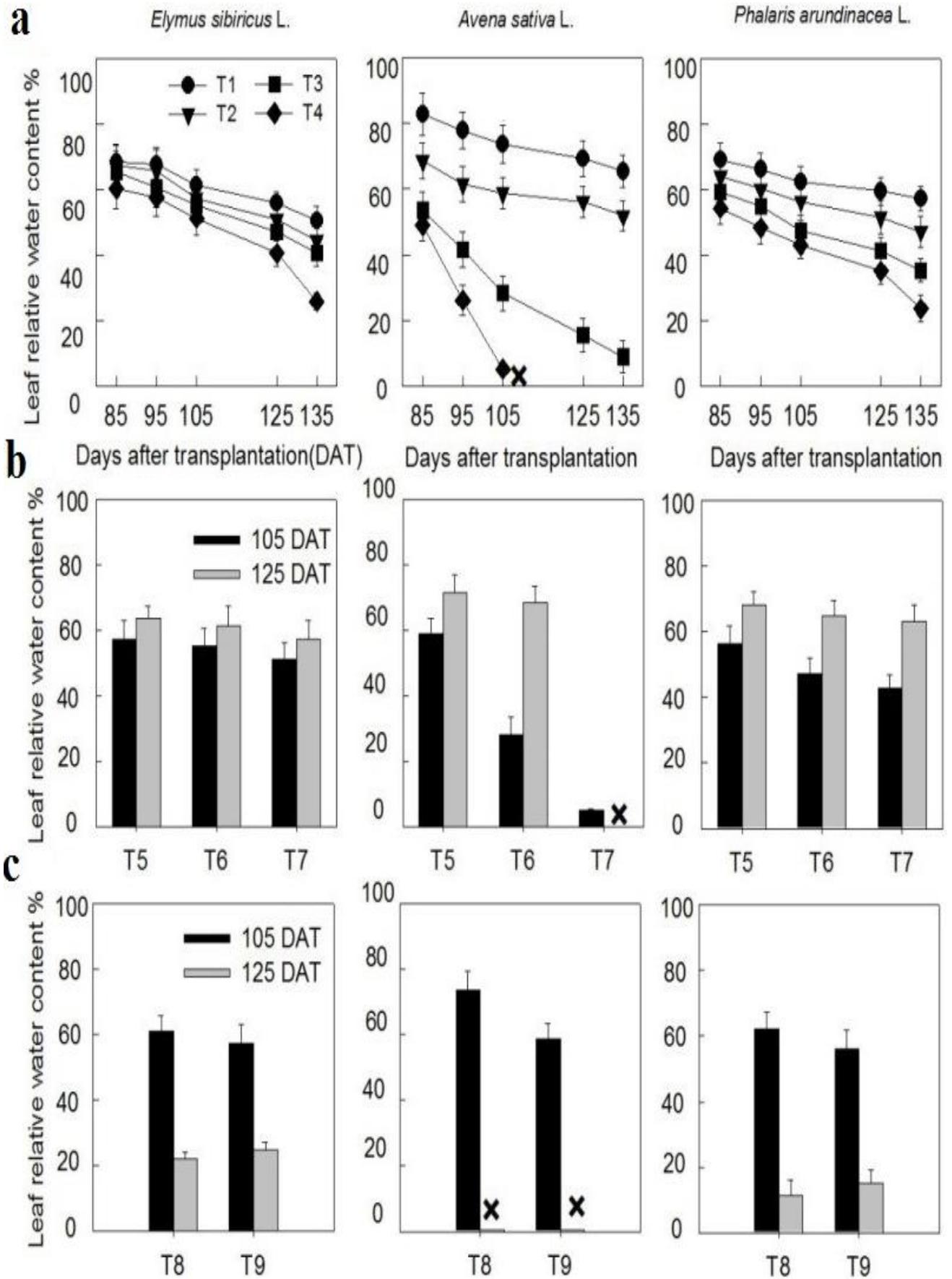


Fig. 7. Relative water content (RWC) in 3 grassland species (*Avena sativa* L., *Elymus sibiricus* L. and *Phalaris arundinacea* L.). Plants were grown at different salt concentrations (Fig. 1), where X was the plant's death. These were measured at five different developmental stages (Mean \pm SE; n = 5).

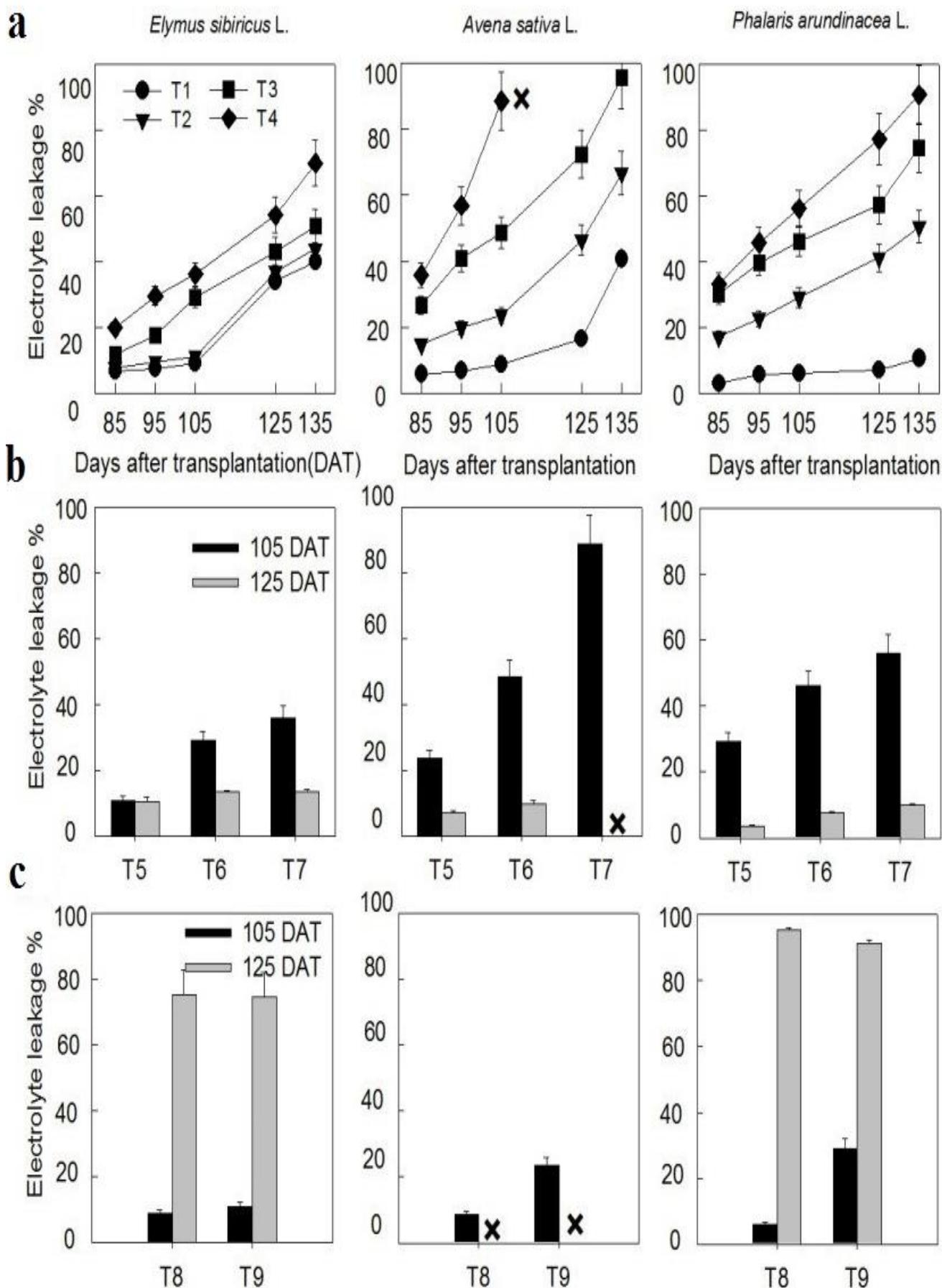


Fig. 8. Electrolyte leakage in 3 grassland species (*Avena sativa* L., *Elymus sibiricus* L., and *Phalaris arundinacea* L.). Plants were grown at different salt concentrations (Fig. 1), where X was the plant's death. These were measured at five different developmental stages (Mean \pm SE; n = 5).

Discussion

Several studies reported that salt stress could affect photosynthesis rates through stomatal closure, damage in photochemical reaction and carbon assimilation (Megdiche *et al.*, 2008; Stoeva & Kaymakanova, 2008). Recently, leaf photosynthesis parameters were used to screen efficient one among six alfalfa cultivars in semi-arid area. High P_n rates and $iWUE$ mean plant can perform better during stress condition (Wang *et al.*, 2017). During increased salt treatment (Fig 1a) and salinization process (Fig 1c) P_n rates and $iWUE$ for *Elymus sibiricus* performed slightly better than *Phalaris arundinacea*. Here, *Avena sativa* could not tolerate high salinity level and died (Fig. 2 & 3a, c). However, during recovery experiment (Fig 1b), *Phalaris arundinacea* significantly increased P_n rates and $iWUE$ followed by *Elymus sibiricus* (Fig. 2 & 3b). Interestingly, recovery rates were opposite to other two increased salt experiments. This may represent that *Phalaris arundinacea* can recover better to certain level of salinity. Noticeably, *Avena sativa* T7 plant could not survive. In our study, salt accumulation negatively affected P_n rates and $iWUE$ for all three species but they respond differently in altered stress conditions. Their response to these parameters screened *Elymus sibiricus* as most tolerant whereas *Phalaris arundinacea* showed better ability to recover under salinity in QTP environment. Our results are consistent with previous findings by (Allakhverdiev *et al.*, 2000; Stepien & Klobus, 2005, 2006), where the highest salinity caused restraint in photosynthesis parameters.

The chlorophyll fluorescence parameters are considered as an important indicator of plants photosynthesis efficiency under different kind of environmental stresses. These are often used to evaluate nitrogen fertilizer uptake and utilization (Wang *et al.*, 2017). Previous studies reported that PSII adversely affected during salt stress and resulted in decline of different chlorophyll fluorescence parameters (Abdeshahian *et al.*, 2010; Gomathi & Rakkiyapan, 2011). In a previous study by Baker (2008), crops cultivars were screened to salt stress tolerance in term of response to PSII efficiency. Here, visual analysis of chlorophyll fluorescence parameters (Fv/Fm and $\Phi PSII$) after 105 day. *Elymus sibiricus* displayed better followed by *Phalaris arundinacea*, whereas *Avena sativa* exhibited least tolerance (Fig. 4a, b) during salinization (Fig. 1a, c). However, *Phalaris arundinacea* exhibited better recovery than *Elymus sibiricus* (Fig. 1b). Further, chlorophyll fluorescence parameters (qP and NPQ) during progressive salt treatment (Fig 1a) and salinization process (Fig 1c) demonstrated more stable nature in *Elymus sibiricus* compared to *Phalaris arundinacea* (Fig. 5 & 6a, c). Although, NPQ results revealed slightly less performance of *Elymus sibiricus* but qP values for *Elymus sibiricus* were significantly higher than *Phalaris arundinacea* in T8 and T9. These results specified *Elymus sibiricus* was more salt tolerant (Fig. 5 & 6c). However, *Avena sativa* plants could not survive to these growing salinity level (Fig. 5 & 6; a, c). Further, during recovery experiment (Fig 1b) *Phalaris arundinacea* performed better compared to *Elymus*

sibiricus (Fig. 5 & 6b). These results expressed *Elymus sibiricus* as more stable species to salt stress. Similar results were described in previous studies where decrease in qP , Fv/Fm and $\Phi PSII$ was increased while NPQ was decreased when plants were exposed to salt stress (Netondo *et al.*, 2004; Jiang *et al.*, 2006; Abdeshahian *et al.*, 2010).

Soil salinity is a key factor limiting crop yield through modification of several physiological and biochemical processes. It is generally believed that plants suffer water deficiency when exposed to salinity, resulting growth inhibition. Plants exposed to salt stress reduce relative water content (RWC) and increase electrolyte leakage (EL). Several previous studies reported that RWC level was reduced more rapidly in salt-sensitive grasses than salt-tolerant under salinity stress (Suplick-Ploense *et al.*, 2002; Alshammery *et al.*, 2004; Liu *et al.*, 2009) and increased in EL, is considered as indication of membrane damage (Simon, 1974). In this study, leaf RWC (Fig. 7) showed the earlier and more severe decline, whereas leaf EL (Fig. 8) showed an earlier and sharper increase in *Avena sativa* compared to *Phalaris arundinacea* and *Elymus sibiricus*. However, *Elymus sibiricus* was found to be the most stable species to salt stress whereas *Phalaris arundinacea* demonstrated excellent performance during recovery compared to other two species.

Conclusion

This study revealed that *Elymus sibiricus* performance was superior at high altitude under salt stress condition by comparing differences in RWC, EL, photosynthesis and chlorophyll fluorescence parameters. These all parameters clearly showed that *Elymus sibiricus* was more stable among three forage grasses under salinity. Further, *Phalaris arundinacea* performance was found better during recovery situation compared to other two species. *Avena sativa* was found to be the most salt sensitive among all three species. We recommend testing of biochemical and molecular mechanisms of these species under salt stress. This study can provide basis to select salt-tolerant forage species in restoration of degraded grassland at high altitude.

Acknowledgments

The research is supported by the National Natural Science Foundation of China (31400211, 31470342, 31570231, 31670235); the National Basic Research Program of China (973 Program) (2015CB150100); and the Development Project of Transgenic Crops of China (2016ZX08009-003-002).

References

- Abdeshahian, M., M. Nabipour and M. Meskarbashee. 2010. Chlorophyll fluorescence as criterion for the diagnosis salt stress in wheat (*Triticum aestivum*) plants. *World Acad. Sci. Eng. Technol.*, 71: 569-571.
- Allakhverdiev, S.I., A. Sakamoto, Y. Nishijama, M. Inaba and N. Murata. 2000. Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. *Plant Physiol.*, 123: 1047-1056.

- Alshammary, S., Y.L. Qian and S.J. Wallner. 2004. Growth response of four turf grasses to salinity. *Agri. Water Manage.*, 66: 97-111.
- Baker, N.R. 2008. Chlorophyll fluorescence: A probe of photosynthesis *In vivo*. *Ann. Rev. Plant Biol.*, 59: 89-113.
- Bilger, W. and O. Björkman. 1990. Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynth. Res.*, 25: 173-185.
- Bossio, D., W. Critchley, K. Geheb, G. Van Lynden and B. Mati. 2007. Conserving soil protecting water. Comprehensive assessment of water management in agriculture: water for food, water for life. Stylus Publishing, LLC, Sterling, VA, pp. 551-584.
- Brandt, J.S., M.A. Haynes, T. Kuemmerle, D.M. Waller and V.C. Radeloff. 2013. Regime shift on the roof of the world: alpine meadows converting to shrub lands in the southern Himalayas. *Biol. Conserv.*, 158: 116-127.
- Chaves, M. 1991. Effects of water deficits on carbon assimilation. *J. Exp. Bot.*, 42: 1-16.
- Chen, B.X., X.Z. Zhang, J. Tao, J.S. Wu, J.S. Wang, P.L. Shi, Y.J. Zhang and C.Q. Yu. 2014. The impact of climate change and anthropogenic activities on alpine grassland over the Qinghai-Tibet Plateau. *Agri. For. Meteorol.*, 189: 11-18.
- Condon, A.G., R.A. Richards, G.J. Rebetzke and G.D. Farquhar. 2002. Improving intrinsic water use efficiency and crop yield. *Crop Sci.*, 42: 122-131.
- Dong, S.K., R.J. Long, Z.Z. Hu, M.Y. Kang and X.P. Pu. 2003. Productivity and nutritive value of some cultivated perennial grasses and mixtures in the alpine region of the Tibetan Plateau. *Grass Forage Sci.*, 58: 302-308.
- Garg, G.K. and O.P. Garg. 1982. Germination and some metabolic characteristics in green gram seedlings as affected by Na₂CO₃ and NaHCO₃. *Biol. Plant.*, 24: 28-33.
- Ge, Z.M., X. Zhou and K.Y. Wang. 2012. Measured and modeled biomass growth in relation to photosynthesis acclimation of a bioenergy crop (Reed canary grass) under elevated temperature, CO₂ enrichment and different water regimes. *Biomass. Bioener.*, 46: 251-262.
- Genty, B., J.M. Briantais and N.R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta.*, 990: 87-92.
- Gomathi, R. and P. Rakkiyapan. 2011. Comparative lipid peroxidation, leaf membrane thermostability, and antioxidant system in four sugarcane genotypes differing in salt tolerance. *Int. J. Plant Physiol. Biochem.*, 3 (4): 67-74.
- He, Y., H. Zhou, X. Zhao, D. Lai and J. Zhao. 2008. Alpine grassland degradation and its restoration on Qinghai-Tibet Plateau. *Pratacul. Ani. Husb.*, 11: 1-9.
- Jiang, Q., D. Roche, T.A. Monaco and S. Durham. 2006. Gas exchange, chlorophyll fluorescence parameters and carbon isotope discrimination of 14 barley genetic lines in response to salinity. *Field Crops Res.*, 96: 269-278.
- Krasilnikov, P., O. Makarov, I. Alyabina and F. Nachtergaele. 2016. Assessing soil degradation in northern Eurasia. *Geoderma Reg.*, 7: 1-10.
- Läuchli, A. and E. Epstein. 1990. Plant responses to saline and sodic conditions. In K.K. Tanji (ed). *Agricultural salinity assessment and management*, ASCE New York., 71: 113-137.
- Liu, S.Z., L. Zhou, C.S. Qiu, J.G. Zhang, Y.P. Fang and W.S. Gao. 1999. *Studies on grassland degradation and desertification of Naqu Prefecture in Tibet Autonomous Region*. Tibet People's Press, Lasha (In Chinese).
- Liu, Y., F. Cheng, Q. Wang, Y. Hu, and Z. Wang. 2009. Salinity stress responses and thresholds in four warm season turf grasses. *Acta Prat. Sin.*, 18: 192-199.
- Long, R.J. 1995. Seasonal dynamics of nutrient metabolites in serum of grazing Yak on alpine grassland. Ph.D. thesis, Gansu Agricultural University, Lanzhou, China.
- Lu, B.R. 1993. Biosystematic Investigations of Asiatic Wheatgrasses *Elymus* L. (Triticeae: Poaceae). Ph.D. Thesis, The Swedish University of Agricultural Sciences, Svalöv, Sweden.
- Ma, Y., Z. Zhang, Q. Dong, J. Shi, Y. Wang and L. Sheng. 2007. Application of restoration ecology in-black soil type degraded grassland rebuilding. *J. Gansu Agri. Uni.*, 42(2): 7-16.
- Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence - a practical guide. *J. Exp. Bot.*, 51: 659-668.
- Megdiche, W., K. Hessini, F. Gharbi, C.A. Jaleel, R. Ksouri and C. Abdelly. 2008. Photosynthesis and photosystem-efficiency of two salt adapted halophytic seashore *Cakile* maritime ecotypes. *Photosynth.*, 46: 410-419.
- Munns, R. 2005. Genes and salt tolerance: Bringing them together. *New Phytol.* 167: 645-663.
- Munns, R., R.A. James and A. Läuchli. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.*, 57: 1025-1043.
- Naseer, M., M. Hameed, A. Zahoor, F. Ahmad, S. Fatima, M.S.A. Ahmad, K.S. Ahmad and M. Iftikhar. 2017. Photosynthetic response in Buttonwood (*Conocarpus erectus* L.) to salt stress. *Pak. J. Bot.*, 49 (3): 847-856.
- Netondo, G.W., J.C. Onyango and E. Beck. 2004. Sorghum and salinity: I. response of growth, water relations and ion accumulation to NaCl salinity. *Crop Sci.*, 44: 797-805.
- Pearcy, R.W. and S.L. Ustin. 1984. Effects of salinity on growth and photosynthesis of three California tidal marsh species. *Oecologia*, 62: 68-73.
- Qin, J., W.Y. Dong, K.N. He, J. Liu and Z.L. Wang. 2010. Physiological responses to salinity in Silver buffaloberry (*Shepherdia argentea*) introduced to Qinghai high-cold and saline area, China. *Photosynth.*, 48 (1): 51-58.
- Qin, Y., S. Yi, S. Ren, N. Li and J. Chen. 2014. Responses of typical grasslands in a semi-arid basin on the Qinghai-Tibetan Plateau to climate change and disturbances. *Environ. Earth Sci.*, 71 (3): 1421-1431.
- Seemann, J.R. and C. Critchley. 1985. Effects of salt stress on the growth, ion content, stomatal behaviour, and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. *Planta.*, 164: 151-162.
- Simon, E.W., 1974. Phospholipids and plant membrane permeability. *New Phytol.*, 73: 377-420.
- SMB (Sichuan Meteorological Bureau) (undated) Compiled meteorological data of Sichuan for the period 1950-1980. Sichuan Meteorological Bureau, Chengdu.
- Stepien, P. and G. Klobus. 2005. Antioxidant defense in the leaves of C₃ and C₄ plants under salinity stress. *Physiol. Plant.*, 125: 31-40.
- Stepien, P. and G. Klobus. 2006. Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. *Biol. Plant.*, 50: 610-616.
- Stoeva, N. and M. Kaymakanova. 2008. Effect of salt stress on the growth and photosynthesis rate of bean plants. *J. Cent. Eur. Agric.* 9: 385-392.
- Sun, H.L. (ed.). 2005. *Ecosystems of China*. Beijing, China: Science Press.
- Suplick-Ploense, M.R., Y.L. Qian and J.C. Read. 2002. Relative NaCl tolerance of Kentucky bluegrass texas bluegrass and their hybrids. *Crop Sci.*, 42: 2025-2030.
- Tang, H., D.W. Zhang, S. Yuan, F. Zhu, F. Xu, F.Q. Fu. 2014. Plastid signals induce alternative oxidase expression to enhance the cold stress tolerance in *Arabidopsis thaliana*. *Plant Growth Regul.*, 74: 275-283.

- Tanka, P.K., E. Lars and K. Sandhya. 2013. Biomass yield and greenhouse gas emissions from a drained fen peatland cultivated with Reed Canary Grass under different harvest and fertilizer regimes. *Bioenerg. Res.*, 6: 883-895.
- Von, C. and G.D. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*, 153(4): 367-387.
- Wang, J., H.J. Huang, S. Jian, X.M. Zhong, F.H. Li, K.G. Zhang and Z.S. Shi. 2017. Photosynthesis and chlorophyll fluorescence reaction to different shade stresses of weak light sensitive maize. *Pak. J. Bot.*, 49(5): 1681-1688.
- Wu, G.L. 2007. The status of oat in the course of sustainable development of stock breeding in alpine meadow. *J. Herbage Feed.*, 1: 10-12.
- Yan, J.J., S.Q. Bai, X. Ma, Y.M. Gan and J.B. Zhang. 2007. Genetic diversity of *Elymus sibiricus* and its breeding in China (In Chinese with English abstract). *Chin. Bull. Bot.*, 24: 226-231.
- Yang, Z.P., J.X. Gao, L. Zhao, X.L. Xu and H. Ouyang. 2013. Linking thaw depth with soil moisture and plant community composition: effects of permafrost degradation on alpine ecosystems on the Qinghai-Tibet Plateau. *Plant Soil*, 367 (1-2): 687-700.
- Yao, T.D., X.D. Liu, N.L. Wang. 2000. Amplitude of climatic changes in Qinghai-Tibetan Plateau. *Chin. Sci Bull.*, 45: 1236-1243.
- Yarsi, G., O. Altuntas, A. Sivaci and H.Y. Dasgan. 2017. Effect of salinity stress on plant growth and mineral composition of grafted and ungrafted Galia C8 melon cultivar. *Pak. J. Bot.*, 49(3): 819-822.
- Zhang, C., D.W. Zhang, Y.N. Sun, M. Arfan, D.X. Li, J.J. Yan, M.H. You, S.Q. Bai and H.H. Lin. 2017. Photo-protective mechanisms in reed canary grass to alleviate photoinhibition of PSII on the Qinghai-Tibet Plateau. *J. Plant Physiol.*, 215: 11-19.
- Zhang, C., Z.M. Ge and X. Zhou. 2013. Effects of elevated CO₂ and temperature on biomass growth and allocation in a boreal bioenergy crop (*Phalaris arundinacea* L.) from young and old cultivations. *Bioenerg. Res.*, 6: 651-662.
- Zhang, J.Y., D.E.C.M.H. Cruz, I. Torres-Jerez, Y. Kang, S.N. Allen, D.V. Huhman, Y. Tang, J. Murray, L.W. Sumner and M.K. Udvardi. 2014. Global reprogramming of transcription and metabolism in *Medicago truncatula* during progressive drought and after rewatering. *Plant Cell Environ.*, 37: 2553-2576.
- Zhang, Y.Q., Y.H. Tang, J. Jiang and Y.H. Yang. 2007. Characterizing the dynamics of soil organic carbon in grasslands on the Qinghai-Tibetan Plateau. *Sci. China Ser. D.*, 50(1): 113-120.
- Zhao, X. 2011. Grassland ecosystem restoration and sustainable management of the source region of three river. Beijing: Science Press.

(Received for publication 27 March 2018)