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Linkage between leaf development and photosynthetic response at hyperosmotic salinity in the C-4 grass *Panicum antidotale*

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ABSTRACT

This study aimed at elucidating the impact of salinity on the differential responses of the photosynthetic apparatus at different stages of leaf development in the C4 perennial grass *Panicum antidotale*. Plants were grown for a period of 15 days at hyper-osmotic salinity (up to 400 mM NaCl) or at control conditions (no addition of NaCl to the culture medium). Each day plants were harvested and salt stress effect on biomass production (fresh and dry weight) was recorded. In parallel leaves were divided from leaf tip to leaf base in five segments for analysis of stress effects on the following parameters: chlorophyll fluorescence parameters (Fv/Fm, ΦPSII, ETR), MDA (an indicator of peroxidation of membrane lipids), and ion leakage.

A direct correlation between membrane lipid peroxidation and (i) membrane integrity as well as (ii) inhibition of PSII function was found under salinity. In monocots the leaf meristem is located at the base and mature parenchymatic cells preferentially are found at the leaf tip. This may explain our finding that the degree of salt mediated inhibition of photosynthetic activity ($ETR_{control}/ETR_{stressed}$) was 20-fold higher at leaf tip than at leaf base. Thus, we discuss observed variance in apparent salt resistance in terms of heterogeneity of the photosynthetic apparatus (due to difference in spatial and temporal development) along the leaf lamina.

1. Introduction

Physiological and biochemical processes are disturbed at enhanced salinity and often lead to a significant reduction in plant biomass production or even reduce survival rate (Flowers and Colmer, 2008). Photosynthesis is known to be very sensitive to salinity because of osmotic effects and ion toxicity (Koyro et al., 2013; Redondo-Gómez et al., 2010). Stomatal conductance, C- and N-assimilation rate, and efficiency of absorbed light energy use are established parameters to evaluate the level of stress (Ahmed et al., 2013; Koyro et al., 2013). Thus, detailed analysis of photochemical reactions may provide insight in the exact physiological, metabolic, and regulatory mechanisms resulting in the observed growth inhibition under salinity.

In contrast to typical anatomy of dicot leaves, in monocot leaves meristematic cells are preferentially found at the leaf base (Baumgartner et al., 1989; Conklin et al., 2019). Therefore, intermediate stages of cell differentiation from young undeveloped cells to mature cells can be analyzed in leaf sections. Early stages will be found near the leaf meristem, while the fully developed system will be found at the leaf tip (Li et al., 2010; Sharman, 1942; Yu et al., 2015). Leaf lamina is preferentially extended by turgor controlled cell extension in a zone following the basal zone of cell division (Nelson and Langdale, 1989). In this zone chloroplast number per cell is multiplying independently of cell division (Martineau and Taylor, 1985). In young leaves of C4 grasses a basal zone of cell division, a zone of leaf cell expansion, and a zone of fully developed cells near the leaf tip are found as well (Majeran and Van Wijk, 2009), but the situation becomes more complicated, as the typical Kranz anatomy of a C4 leaf develops in parallel to cell development. Development of leaf anatomy is paralleled in a C4-leaf by modification of the compartmentation of metabolic pathways and metabolite transport activity (Preiss et al., 1994; Yang et al., 2008). At the molecular level, the C4 leaf differentiation includes transcriptional regulation through DNA regulatory elements, transcription factors and metabolic signals (Edwards et al., 2001; Nelson and Langdale, 1992; Sheen, 1999). Sugar and sugar phosphate signaling is involved in the control of leaf development (De Souza et al., 2018). Therefore, it may be expected that salinity may affect successively photosynthesis, namely (i) energy dissipation (heat production, futile cycles including ROS production), (ii) chlorophyll fluorescence, and (iii) photosynthetic electron transfer (Ashraf and Harris, 2013; Kocheva

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et al., 2004) but also the sugar metabolism and leaf development (Majeran and Van Wijk, 2009). As a rule, salinity induced effects on the photosynthetic activity are measured without considering heterogeneity of leaves (Chaerle et al., 2009). However, there are reports describing plasticity of observed stress responses based on parameters, such as spatial and developmental effects (Ahmed et al., 2013; Chaerle et al., 2009; Martinez-Penalver et al., 2011; Oxborough, 2004; Zribi et al., 2009).

In the present study, we have chosen an NADP-ME C4 type monocot *Panicum antidotale* (Joseph, 2005; Siebke et al., 2003). The C4 photosynthetic pathway is more energy-requiring than the C3 pathway. This extra energy is associated with the regeneration of phosphoenolpyruvate by the C4 cycle in the mesophyll cells (Krall and Edwards, 1992). Therefore, one would assume that it might adversely affect salt resistance. However, it has been found that *P. antidotale* is able to grow and produce biomass in up to 500 mM NaCl (100% sea water salinity) (Koyro et al., 2013). The combination of C4 specific leaf development, its impact on photosynthesis, the expected higher energy demand in saline environments, the high salt resistance and the economic potential in semiarid areas of *P. antidotale* are ideal preconditions to introduce this species as a model plant. Elucidating the response of plants to saline environments is of interest to people of many disciplines practiced in semi-arid areas.

It was the aim of this study to correlate salinity and leaf development with reliable parameters such as differences in the sequence and the timing of biochemical reactions and photosynthetic activity in *Panicum* leaves. Therefore, we have tested the hypothesis that growth and photosynthetic response to salinity are based on tissue development gradient of the leaf. We also hypothesized that tissues located at or near leaf tip would face severe salt stress.

2. Materials and methods

2.1. Growth culture

Seeds of *Panicum antidotale* Retz. (collected from a mono-specific stand located near Hub, Pakistan, 24° 58′ 17.28″ N, 66° 46′ 33″ E) were germinated on TKS1 substrate (Floragard, Oldenburg, Germany) in a growth chamber at a 14:10 h day light : night cycle at 25 °C. Ten-days-old seedlings were transferred to the greenhouse and left to acclimatize for another 15 days at day / night temperatures of 24 °C/20 °C, about 60% humidity, and 16 h of light period per day. A constant quantum flux rate of 300 µmol photons m^{-2} s^{-1} was achieved by additional illumination using sodium vapor lamps, SON-T Agro 400, Philips, Hamburg, Germany. Seedlings (3–4 leaf stage) were transferred to 1 L plastic pots (20 cm × 25 cm) filled with sand. The plants were irrigated with Epstein's (Epstein, 1972) growth medium. After a period of 2 weeks salinity of the solution was raised in steps of 50 mM added NaCl per day until the final concentrations of treatments (0 (control), 100, 200, 300 and 400 mM NaCl) were reached. Each day water was added to correct for evaporation. After passage through the sand, solutions were collected in 50 L buckets and recycled for irrigation. Growth media were changed every week to avoid nutrient depletion. There were two sets of such salinity treatment experiments. Each of them contained 4 biological replicates per treatment. Plants were harvested 15 days after reaching the final salinity stage.

In order to analyze responses to extreme stress in more detail 3 biological replicates representing 3–4 leaf staged seedlings with leaf length of about 7–10 cm were used in a second experiment. In this set up, plants were harvested each day after completion of the final salinity level for a period of 15 days. Control plants were compared to plants treated with 400 mM NaCl added to Epstein's medium.

2.2. Final harvest

Plants were divided into shoot (aboveground) and root (underground). Fresh weight was measured immediately after harvest while dry weight was determined after drying the plants in an oven at 70 °C till a constant weight was obtained. The shoot height (length of aboveground plant from the base to the tip of the stem, i.e. where highest leaf inserted) was recorded after 15 days in the first set of experiments and daily in the second set of experiments focusing on spatiotemporal parameters. All data were expressed on per plant basis.

2.3. Chlorophyll content

Concentrations of chlorophyll *a* (Chl a), chlorophyll b (Chl b) and total chlorophyll were analyzed according to Lichtenthaler (1987) on both sets of experiments. Every day from day 0–15, 0.1 g of leaf discs were collected from the middle portions of the healthy leaf from the third nodes of the shoot tip irrespective of being formed before or after onset of treatment. In case of the spatiotemporal experiment we took samples from 6 different plants daily from day 0–15. Therefore, 3 replicates were taken of the control and salt treatment, each adding up to overall 96 plants. The leaf samples were homogenized together with 10 ml of 80% acetone in mortar and pestle. The concentrations of Chl a, Chl b and total chlorophyll were measured using a UV–vis spectrophotometer (Beckman Coulter – DU® 500 UV/Vis Spectrophotometer) at 663.2, 646.8 and 470 nm using quartz cuvettes respectively. A solution of 80% acetone was used as a blank. Concentrations of chlorophyll *a*, chlorophyll b, and total chlorophyll were calculated from the equations as described by Lichtenthaler (1987).

2.4. Chlorophyll fluorescence measurement

Chlorophyll *a* fluorescence parameters were measured on dark adapted (20–30 min) and five selected leaf portions (cf. Fig. 1), using Junior PAM (Walz, Germany). Here, necrotic areas that appeared mainly after 12 days at tip and margin of leaf of 400 mM NaCl treated plants were avoided. The minimal fluorescence (Fo) was recorded after a flash of < 0.1 µmol photon m^{-2} s^{-1} light followed by a saturating pulse of 10,000 µmol photon m^{-2} s^{-1} for 0.6 s to determine maximal fluorescence (Fm), and maximum photochemical quantum yield of PSII [Fv/Fm = (Fm-Fo)/Fm] was estimated. After light adaptation of leaf, steady-state (Fs) and maximal fluorescence (Fm') were recorded and used to calculate effective photochemical quantum yield of PSII as $\sqrt{}_{PSII}$ = (Fm'-Fs)/Fm' (Genty et al., 1989). Photochemical and non-photochemical quenching (qP and NPQ respectively) were estimated as qP = (Fm'-Fs)/(Fm'-Fo') and NPQ = Fm/Fm'-1 (Bilger and Björkman, 1990; Kooten and Snel, 1990; Schreiber et al., 1986). The method of Krall and Edwards (1992) was used to estimate the linear apparent electron transport rate (ETR) based on the energy yield of PSII ($\sqrt{}_{PSII}$). Thus, ETR is calculated as ETR = Φ_{PSII} × PPFD × 0.5 × 0.84, where PPFD is the Photosynthetic Photon Flux Density incident on the leaf; the factor 0.5 is used on the assumption of an equal distribution of energy between the two photosystems; the factor 0.84 represents an assumed leaf absorbance.

Thus, the equation used by us resembles a simplification of the one described by Fryer et al. (1998), which was introduced to evaluate photosynthesis in the C4 plant maize. Fluorescence measuring responses of micro-disc areas with the Junior PAM set up allowed monitoring of spatial and temporal salinity effects on *Panicum* leaves from base (above ligule) to the tip of the leaf and named as segment 1–5 (Fig. 1).

2.5. Electrolyte leakage

Electrolyte leakage content was measured daily using the 3rd and 4th leaf below the shoot tip. Leaf discs (1 cm diameter, 3 replicates) from the healthy (not necrotic spots) of middle leaf portions were incubated in 3 ml of deionized water in a desiccator at 10^{-1} bar for 60 min. Then leaf discs were removed and the conductivity of the water

Fig. 1. *Panicum* leaf showing various segment positions (numbers 1–5) from base to tip at day 15 of the salt treatment (0 and 400 mM NaCl).

was measured (EC_o). In a parallel test, leaf discs incubated in water were heated for 120 min at 95 °C in an autoclave to completely destroy bio-membranes prior to the leakage test. This value represented maximal conductivity (EC_m). The electrolyte leakage of leaf discs from the control and salinity treatments was calculated as a percentage of maximal conductivity ($EL\% = EC_o/EC_m \times 100$) (Dionisio-Sese and Tobita, 1998).

2.6. Determination of the malondialdehyde content in leaves

Malondialdehyde (MDA) content is a well-known indicator for oxidation of lipids associated with cell membranes. Fresh leaf (100 mg) samples (only taken from the middle of the leaf lamina by avoiding the necrosis and limitation of plant material) were homogenized in cold 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) solution. It was boiled in a water bath (95 °C for half an hour) and the reaction terminated immediately on ice followed by centrifugation for 5 min at 3000 × *g*. The absorbance was measured at 532 nm and 600 nm for MDA estimation (Cakmak and Marschner, 1992).

2.7. Statistical analysis

Data were analyzed by one-way ANOVA (Analysis of Variance) in case of first experiment (0, 100, 200, 300 and 400 mM NaCl) and values represent the means and standard errors (n = 4). Bonferroni post-hoc test was also carried out to compare between the treatments. Two-way ANOVA was performed on biomass, chlorophyll, MDA and ion leakage for analyzing the effect of independent factors; salinity (0 and 400 mM NaCl) and time (days 0–15). Repeated Measure ANOVAr was tested on the spatiotemporal experiment for fluorescence parameters to elucidate

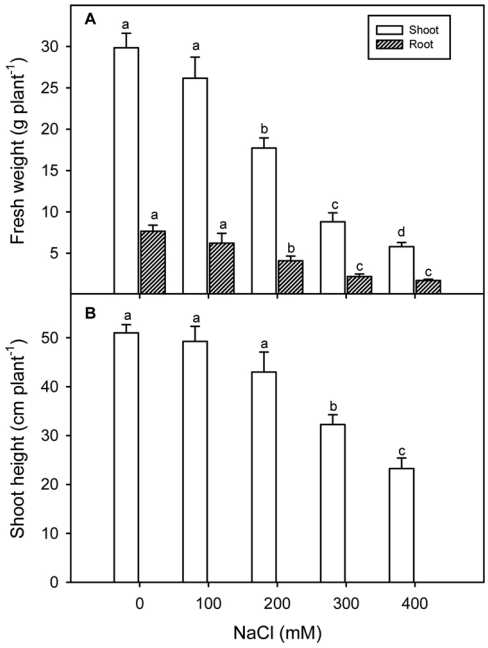

Fig. 2. Growth response: Fresh weight (A) (shoot and root) and shoot height (B) of *Panicum antidotale* in presence of 0, 100, 200, 300 and 400 mM NaCl. Values represent mean ± SE (n = 4). Different letters indicate significant difference between treatments at $P < 0.05$ using a Bonferroni test.

the effect of salinity, time (days) and leaf development (leaf base to tip), where days and leaf segments were selected as the repeated measurements while salinity was selected as between-subjects factor by using the mix approach repeated measure ANOVAr. The values represent means and standard errors (n = 3). All analyses were performed at $P < 0.05$ significance level using SPSS (version 24) for Windows and are presented in supplementary material with detailed description.

3. Results

3.1. Initial experiment

During the experimental period of 15 days biomass production of *Panicum antidotale* remained unchanged in presence of added 100 mM NaCl in comparison to the control. However fresh weight and shoot height significantly ($P < 0.001$) decreased with further increases in salinity (Fig. 2). Chlorophyll concentration decreased significantly ($P < 0.01$) when NaCl concentrations exceeded 200 mM (Fig. 3). Chlorophyll *a* to chlorophyll b ratio was maintained in all salinity treatments. At 400 mM NaCl Fv/Fm, Φ_{PSII} and ETR decreased by 22%, 24% and 24% respectively, as compared to the control. In comparison to non-saline control all salt treatments caused a significant decline of NPQ (Table 1).

3.2. Spatiotemporal experiment

After 15 days of treatment, fresh weight of control plants was 24-fold higher as compared to plants grown in presence of 400 mM NaCl, while dry weight of controls was about 9-fold higher as compared to the salt treated plants (Fig. 4 A–D). Shoot height progressively increased to 2-fold after 5 days and 5-fold after 15 days in control plants as compared to those grown in presence of 400 mM NaCl (Fig. 4E). In addition, we observed, approximately, an increase of leaf length from 7 to 10 cm (initial length) to 20–23 cm (at the end of treatment).

The maximum photochemical quantum yield of photosystem II (Fv/Fm) varied between the 5 analyzed leaf segments. Moreover, changes were observed with plant development as well: (i) After 8 days of salt

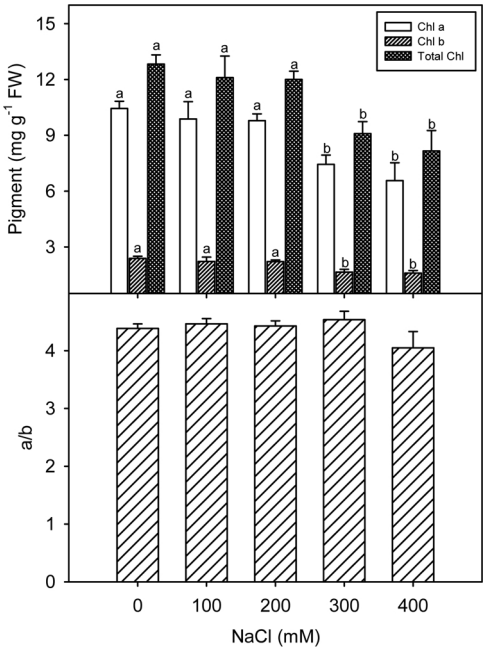

Fig. 3. Analysis of photosynthetic pigments: Chla, Chlb, total Chl and Chl a/b ratios measured in leaves of *Panicum antidotale* grown in presence of 0, 100, 200, 300 and 400 mM NaCl added to the culture medium. Values represent mean ± SE (n = 4). Different letters indicate significant difference between treatments at $P < 0.05$ using a Bonferroni test.

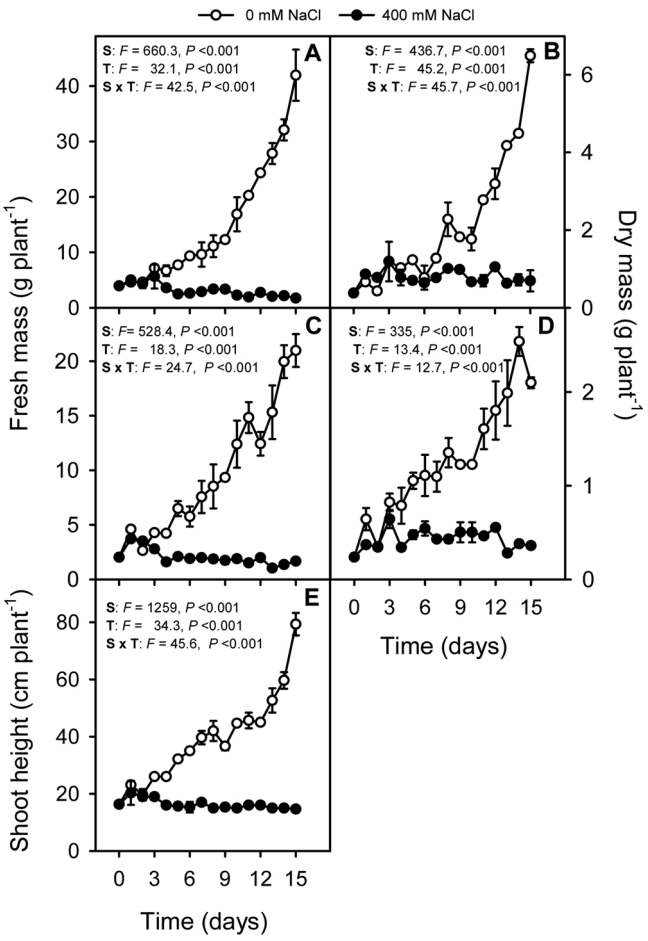

Fig. 4. Growth of *Panicum antidotale* in response to salinity: The variation of stress response with time (days) is shown. Measured parameters are fresh weight of shoot and root (A & C), dry weight of shoot and root (B & D) and height of shoot (E). Values represent mean ± SE (n = 3). - Open circles are showing data of the controls (0 mM NaCl), closed circles are showing data of plants grown in presence of 400 mM NaCl. *F* values and *P* values from two-way ANOVA on salinity (S: 0 and 400 mM NaCl) and time (T: 15 days) at 95% significance level.

treatment, a more pronounced decrease of Fv/Fm in segments 4 and 5 of stressed plants was found as compared to the control than for segments near to the leaf base (Fig. 5A). (ii) By the end of the experimental period Fv/Fm values declined under salt treatment at the leaf base (segments 1) and tip (segments 3, 4 and 5) to about 1/3 and 1/12 respectively, as compared to control plants. (iii) The effective photochemical quantum yield of photosystem II ($\sqrt{}_{PSII}$) was constant in the leaf base segments until day 5 but declined significantly in segments of the leaf tip under NaCl treatment (Fig. 5B). (iv) With increasing duration of salt exposure, $\sqrt{}_{PSII}$ was severely reduced at the leaf tip (down to about 1/15 at day 15) in comparison to control plants. This salinity induced reduction was lower (down to about 1/3 of control leaves until day 15) in segments at the leaf base. (v) The apparent electron transport rate (ETR) was severely reduced (down to about 1/14) in leaf tip segments as compared to the leaf base (down to about 1/3), in salt treated leaves at day 15 compared to control plants (Fig. 5C). (vi) After 5 days of hyperosmotic salinity, a significant decrease of pigment concentration of leaves from stressed plants could be measured for the first time. Visual observation of the leaves showed that chlorosis (yellowing of leaf) was more pronounced at tip and near to tip as compared to base of the leaf (cf. Fig. 1). An even more pronounced difference was found after 7 days. At that incubation time the concentrations of chlorophyll *a* and chlorophyll b were only half as much in plants grown in presence of 400 mM NaCl as found in leaves of control plants. At the end of the experiment the pigment concentrations of leaves from the highest salinity treatment were only one fourth of the controls (Fig. 6).

Stress markers such as ion leakage and MDA content were increased significantly with duration of salt treatment (Fig. 7). Ion leakage in 400 mM NaCl treated plants was 2-, 3- and 6-fold higher as compared to control plants on days 5, 9 and 15, respectively (Fig. 7). MDA content showed a delayed response in comparison to ion leakage. The contents were 1.5- and 3-fold higher at hyperosmotic salinity conditions as compared to controls on days 9 and 15, respectively (Fig. 7).

Table 1
Fluorescence parameters, maximum photochemical quantum yield of photosystem II (Fv/Fm), effective photochemical quantum yield of photosystem II(Φ_{PSII}), non-photochemical quenching (NPQ) and relative electron transport (ETR), measurements on 3rd and 4th node's leaf of *Panicum antidotale* in response to 0, 100, 200, 300 and 400 mM NaCl concentration. Values represent mean ± SE (n = 4). Different letters indicate significant difference between treatments at P < 0.05 using Bonferroni test.

NaCl (mM)	Fv/Fm		Φ_{PSII}		NPQ		ETR	
0	0.766a	± 0.006	0.446a	± 0.024	1.006a	± 0.047	53.42a	± 2.906
100	0.752a	± 0.000	0.450a	± 0.015	0.763b	± 0.012	53.85a	± 1.787
200	0.718ab	± 0.013	0.432a	± 0.026	0.761b	± 0.005	51.65a	± 3.148
300	0.680bc	± 0.012	0.356ab	± 0.048	0.758b	± 0.013	42.65ab	± 5.776
400	0.599c	± 0.034	0.338b	± 0.016	0.759b	± 0.04	40.40b	± 1.892

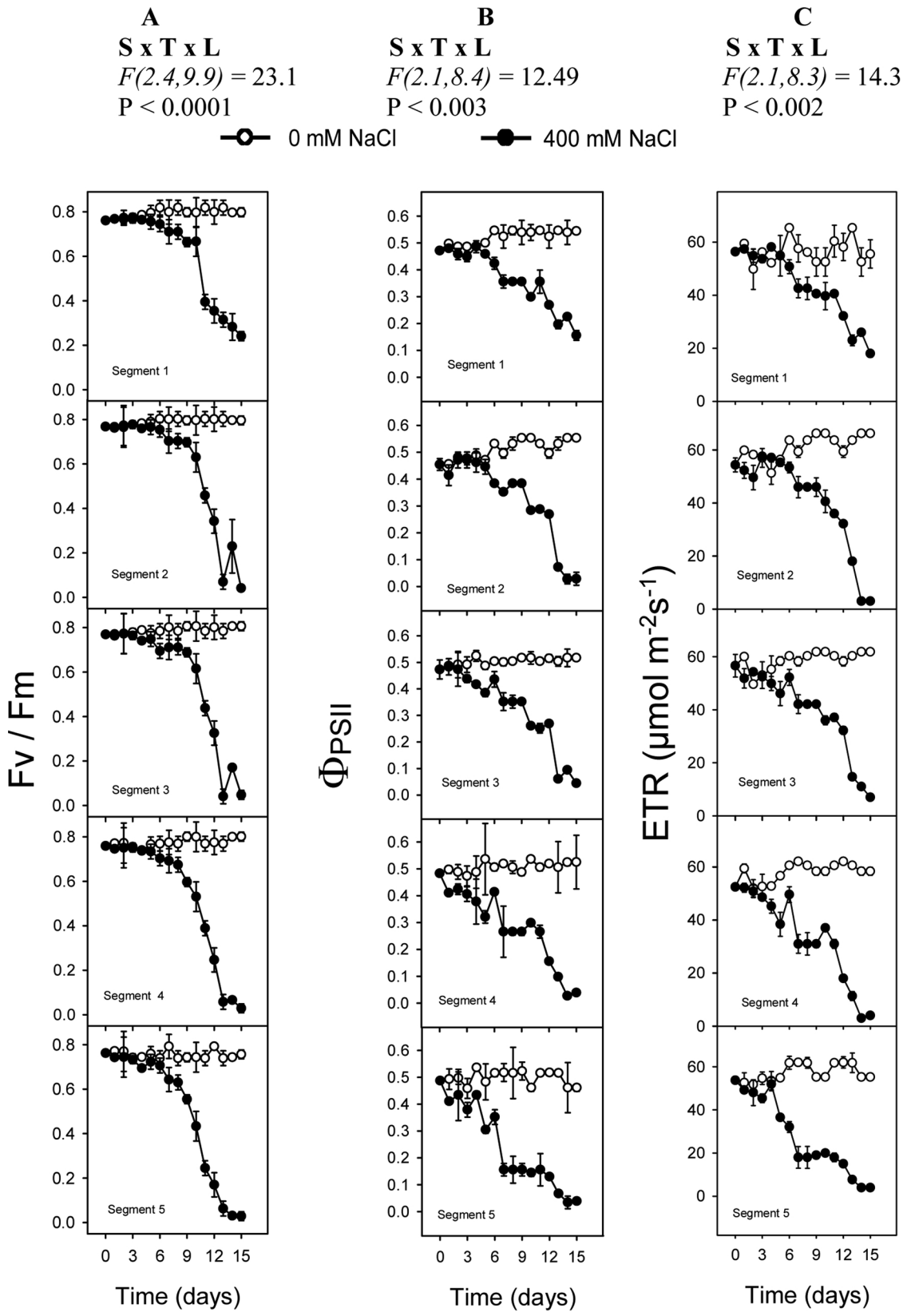

Fig. 5. Variation of fluorescence parameters in response to salinity: Parameters have been analyzed in leaves of *Panicum antidotale* with respect to both, temporal (days) and developmental effects. Open circles show data of the control plants (0 mM NaCl), closed circles indicate data of plants grown in presence of 400 mM NaCl. Five leaf segments (tip to base, as shown in Fig. 1) have been analyzed. The following parameters have been measured: Maximal photochemical quantum yield of photosystem II (Fv/Fm), effective photochemical quantum yield of photosystem II (Φ_{PSII}), and apparent electron transport rate (ETR). Presented data represent mean ± SE (n = 3). *F* values and *P* values from repeated measure ANOVAr on salinity (S: 0 and 400 mM NaCl), time (T: 15 days), and leaf lamina from base to tip (L: 1–5 segment) at 95% significance level, where leaf segment and time (days) were selected as repeated parameter while salinity was a fixed factor.

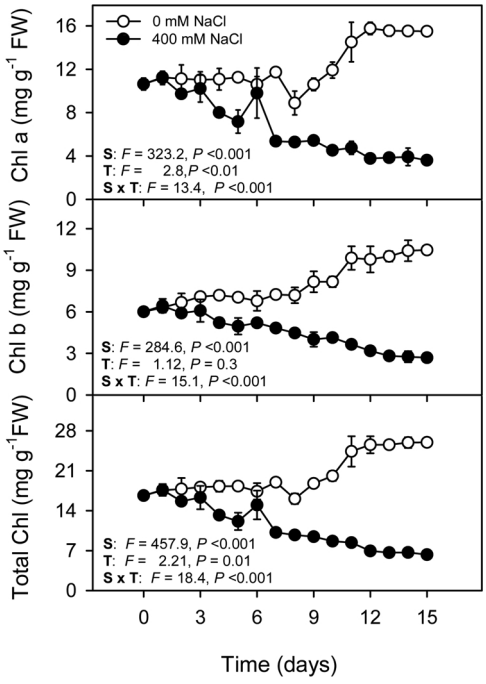

Fig. 6. Analysis of photosynthetic pigments: Salinity effects on Chl a, Chl b, and total Chl contents have been monitored during a period of 15 days in leaves of *Panicum antidotale* (open circles: control plants, close circles: plants cultivated in presence of 400 mM NaCl). Values represent mean ± SE (n = 3). *F* values and *P* values from two-way ANOVA on salinity (S: 0 and 400 mM NaCl) and time (T: 15 days) at 95% significance level.

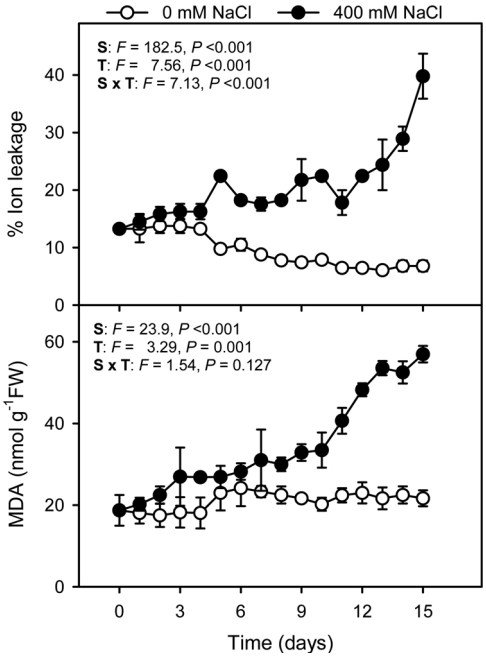

Fig. 7. Stress markers (ion leakage and MDA content) in the leaves of *Panicum antidotale:* Salinity effects on stress markers (ion leakage and MDA content) have been monitored for a period of 15 days (open circles: control plants; closed circles: plants cultivated in presence of 400 mM NaCl). Values represent mean ± SE (n = 3). *F* values and *P* values from two-way ANOVA on salinity (S: 0 and 400 mM NaCl) and time (T: 15 days) at 95% significance level.

4. Discussion

Growth of *Panicum antidotale* was increasingly hindered with duration of exposure to hyperosmotic salinity (400 mM NaCl). In agreement with the results shown in this paper, several authors described the regulation of plant development and growth by various physiological and biochemical processes (Koyro et al., 2013, and citations therein). These included (i) stress effects on leaf development (Mullet, 1988; Tsutsumi et al., 2014; Yuan et al., 2015) as well as (ii) early stress responsive reactions of photosynthesis (Benzarti et al., 2012; Covshoff et al., 2014; Kutschera et al., 2010; Nesterenko et al., 2006; Pantin et al., 2012).

The purpose of the present study was to analyze in parallel the impact of salinity on single leaf development and photosynthetic activity. First of all, it was observed that salt-induced changes in growth were paralleled by distinct changes in the photosynthetic pigment pattern. As shown in Figs. 1 and 2, increasing salinity resulted in a significant reduction of plant growth. This inhibition was found with both parameters, shoot height and biomass production. With respect to leaf growth, measured parameters do not allow to tell whether inhibition was due to an inhibition of cell division in basal leaf segments or an inhibition of turgor mediated cell extension in the differentiating leaf region. However these processes, obviously, reduce plant photosynthetic capacity under stress conditions (Jiang and Rodermel, 1995; Kutschera et al., 2010; Parida et al., 2004). In addition, chlorophyll biosynthesis in developing leaves under stress may need further explicit studies to answer whether the change of pigments is due to damage in chloroplast number or some restriction in chlorophyll biosynthesis during cell differentiation under salt stress.

As outlined in the literature, chlorophyll b is preferentially present in the light harvesting complexes, while the core complexes of the reaction centers of the photosystems I and II exclusively contain chlorophyll *a*. Therefore, changes of chlorophyll *a*:*b* ratios would reflect modifications in the light harvesting antenna (see Nixon et al., 2010, and citations therein). As documented in Fig. 3, a reduction in chlorophyll *a*:b ratio was observed only under severe stress (a statistically non-significant reduction), when salinity exceeded 300 mM NaCl. On the other hand, as shown in Fig. 3, total chlorophyll concentration of leaves was decreasing with increasing salinity. In presence of mild salinity stress (at NaCl concentrations below 400 mM) chlorophyll *a*:b ratio remained constant. This observation is supporting our above mentioned statement that moderate salinity results in a reduction of chlorophyll biosynthesis (Dalal and Tripathy, 2012; Holland et al., 1998; Jiang and Rodermel, 1995) and, thus, a potential reduction in the efficiency of light absorbance.

Reduction of chlorophyll concentration commonly is used as an indicator of plant stress sensing. It can be attributed to (i) abundance of chloroplasts per leaf area, (ii) chlorophyll degradation, as well as (iii) inhibition of chlorophyll synthesis (Holland et al., 1998; Pirada et al., 2004; Tezara et al., 2003). Reduced leaf chlorophyll concentration will affect three pathways competing for energy consumption, namely (i) energy dissipation (heat production, futile cycles including ROS production), (ii) chlorophyll fluorescence, and (iii) photosynthetic electron transfer (Ashraf and Harris, 2013; Kocheva et al., 2004). Reduced abundance of chlorophyll therefore can be beneficial if photosynthetic energy use is inhibited (Geissler et al., 2009). In such a situation the probability of a direct electron transfer from activated chlorophyll to molecular oxygen, the formation of ROS, otherwise would increase (Foyer and Noctor, 2009).

This interpretation correlates nicely with changes in chlorophyll fluorescence parameters. At day 8, a progressive stress induced decrease in $\sqrt{}_{PSII}$ yield became significantly detectable for the first time in 400 mM NaCl treated plants as compared to control plants. This reduction was accompanied by a decrease of electron transport capacity of stressed samples. A similar pattern of responses has been reported already from experiments with other species (Baker, 2008; Benzarti

et al., 2012; Martínez-Peñalver et al., 2011; Valeur, 2013). The observed damage of $\sqrt{}_{PSII}$ photochemistry indicates salt-induced alterations of thylakoid membrane architecture that may include light harvesting protein complexes as well (Ferroni et al., 2007; Porcar-Castell et al., 2014).

As outlined in the literature, Fv/Fm ratio is sensitive to salt and other stresses (Baker, 2008; Martínez-Penalver et al., 2011). The Fv/Fm values decreased substantially at the leaf tip (segments 4 and 5) of *P. antidotale* and this observation was accompanied by reduction of the chlorophyll concentration and the appearance of chlorosis symptoms (Fig. 3), while a delay in chlorosis as well as less destruction of $\sqrt{}_{PSII}$ was observed at the leaf base. These observations are in agreement with data from the literature (Donnini et al., 2013; Raschke et al., 1990; Sperdouli and Moustakas, 2012). Thus, it may be argued that hyperosmotic salinity is not only affecting leaf tissue development but is also shortening the life span by inducing early senescence of leaf tissues as well as signal transduction between young and mature tissues for stress adjustment between young and mature tissues (Pantin et al., 2012).

As could be expected, the effective photochemical quantum yield of photosystem II ($\sqrt{}_{PSII}$) mirrored the responses of Fv/Fm in *P. antidotale* leaves. It was argued in the literature that the pigment patterns and the orientation of light harvesting and core complexes ($LHCP_{II}$-PS_{II}) vary with chloroplast and leaf tissue development (Covshoff et al., 2008; Izumi et al., 2017; Jiang and Rodermel, 1995; Kutschera et al., 2010; Smillie and Nott, 1982). Moreover, Voznesenskaya et al. (2004) pointed out that they have observed a developmental differentiation in leaves with time. At an early stage of leaf development, leaves apparently lack C4 characteristics (Majeran and Van Wijk, 2009), and cellular partitioning of C3 and C4 specific enzymes could not be detected (Ghannoum et al., 1998; Hasan et al., 2006; Omoto et al., 2010). Respective compartmentation of metabolic pathways as well as development of Kranz anatomy was found only at a later stage of development. Abundance of C4 cell differentiation was starting in tissues of the cell extension zone and was increasingly progressing to the leaf tip (Koteyeva et al., 2014).

This implies variation in fluorescence quenching capacity during development and tissue differentiation. This interpretation is in line with the observation that the apparent electron transport rate (ETR) increased with differentiation of leaf tissues. Beside this variation and in line with findings of other scientists, we could verify the impact of salinity on the differentiation of photosynthesis (see Fig. 5; Jajoo, 2013; Naidoo et al., 2012; Sperdouli and Moustakas, 2012; Zheng et al., 2009).

Consequently, ETR in the tip sections was significantly more sensitive to salinity than ETR in sections at the leaf base. Other fluorescence data are in line with the interpretation that stress sensitivity is under developmental control. However, the measured data do not rule out other interpretations. It may be argued, for instance, that our observations are generated by stress responsive energy sinks in developing leaf tissues (Majeran and Van Wijk, 2009; Wang et al., 2014). These developmental stages in *Panicum*, particularly for leaf, were influenced by salt treatment which is also in agreement with previous studies on salt resistant plants (Debez et al., 2001; Megdiche et al., 2007; Smillie and Nott, 1982). Regarding our results, this implies the conclusion that this difference in sensitivity exists also between zones that are differing in developmental stage in an individual leaf of *Panicum*.

The shown variation in fluorescence quenching capacity at hyperosmotic salinity fits nicely with the reduction of Rubisco protein synthesis in *Panicum antidotale* (Koyro et al., 2013). This effect was paralleled by a reduced CO_2 assimilation activity. Such a reduction of CO_2 assimilation will considerably decrease ATP and NADPH utilization. This reduction in energy demand will lead to a feedback inhibition of the photosynthetic electron transport rate. This in turn results in an increased risk of ROS production, unless (i) absorbed light energy can be dissipated by alternative pathways or (ii) electrons released from activated chlorophyll can be used by futile cycles (Foyer and Shigeoka, 2011; Kreslavski et al., 2013; Moradi and Ismail, 2007). Accordingly, *Panicum* leaves underwent photoinhibition when plants were grown under salinity (Bose et al., 2014; Koyro et al., 2013). This line of arguments was supported in our experiments by chlorophyll fluorescence data as well as by indirect measurement of ROS damage (Fig. 7).

A concerted reduction of the chlorophyll concentration and ETR as shown for *Panicum* could be useful or essentially needed if dissipation of energy by heat release is not available. However, the involvement of heat release in stress avoidance could not be observed by the chlorophyll fluorescence parameter NPQ (Table 1). There are contradictory reports concerning NPQ activity in *Panicum* leaves; while Tezara et al. (2003); Zribi et al. (2009) and Redondo-Gómez et al. (2010) did not find such changes, the opposite result was published by Mateos-Naranjo et al. (2008); Naumann et al. (2008); Benzarti et al. (2012) and Duarte et al. (2013). This discrepancy in results may be due to different developmental stages of the plants or leaf sections analyzed by these research teams.

An efficient oxidative stress avoidance system can be easily proven. Lipid peroxidation, i.e. using malondialdehyde production (MDA) as an indicator, is widely used as a measure of oxidative stress (Adnan et al., 2016; Parveen et al., 2017; Xu et al., 2013). Damage in cell membrane permeability (indicated by ion leakage; Fig. 7), increased during NaCl treatment in *Panicum* as well. This correlation is a typical indicator of ROS mediated membrane damage. We therefore argue that the observed photoinhibition was due to stress induced ROS production.

5. Conclusions

In conclusion, photosynthetic parameters varied with salinity concentration, time of exposure to salt stress and location of the analyzed cells within the leaf lamina (Lehmeier et al., 2017). Under salinity, chlorophyll fluorescence parameters at the leaf tip were inhibited earlier and more severe in comparison to those measured in the leaf base region. Under salt treatment reduction of Fv/Fm and $\sqrt{}_{PSII}$ values were paralleled by a decline in production of plant biomass. Along the leaf lamina from base to tip different degrees of changes in stress mediated chlorophyll fluorescence responses were observed. The variation in response of measured parameters at different points of the leaf lamina mirrors the development of leaf tissues along a gradient extending from the leaf base to the leaf tip; nevertheless, more evidences (e.g. gene and protein expressions) at various ontogenetic stages of leaf development will strengthen our hypothesis. However, the present study underscores the fact that respective measurements of leaf homogenates will not illustrate the complete physiological stress response.

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