

Special issue in honour of Prof. Reto J. Strasser

JIP-test as a tool to identify salinity tolerance in sweet sorghum genotypes

A. RASTOGI*, M. KOVAR**, X. HE***,†, M. ZIVCAK**, S. KATARIA#, H.M. KALAJI##,
M. SKALICKY###, U.F. IBRAHIMOVA†, S. HUSSAIN††, S. MBARKI†††, and M. BRESTIC**.,###,†

*Laboratory of Bioclimatology, Department of Ecology and Environment Protection, Poznan University of Life Sciences, Piątkowska 94, 60-649 Poznań, Poland**

*Department of Plant Physiology, Slovak University of Agriculture, A. Hlinku 2, 94976 Nitra, Slovak Republic***

*Jiangsu Academy of Agricultural Sciences, 210014 Nanjing, China****

School of Biochemistry, Devi Ahilya University, Khandwa Road, 452001 Indore, India#

Department of Plant Physiology, Faculty of Agriculture and Biology, Warsaw University of Life Sciences WULS-SGGW, 159 Nowoursynowska Str., 02-776 Warsaw, Poland##

Department of Botany and Plant Physiology, Faculty of Agrobiological Sciences, Food and Natural Resources, Czech University of Life Sciences, 16500 Prague, Czech Republic###

Institute of Molecular Biology and Biotechnology, Azerbaijan National Academy of Sciences, Matbuat Avenue 2A, Baku, Az 1073, Azerbaijan Republic†

College of Agronomy, Sichuan Agricultural University, 211 Huimin Road, Wenjiang District, 611130 Chengdu, China††

National Research Institute of Rural Engineering, Water and Forests, Aryanah, Tunisia†††

Abstract

The effect of salinity on primary photochemical reactions (using JIP-test) in six sweet sorghum genotypes was tested. An increase in salt concentrations induced significantly the accumulation of proline and caused a decline in leaf osmotic potential. Except for 100 mM NaCl concentration, salinity significantly decreased chlorophyll content and photosynthetic efficiency of plants. Increasing salinity led to a higher accumulation of Q_B -nonreducing PSII reaction centers. K-step in OJIP fluorescence transient was observed for the most sensitive genotypes under the high NaCl concentration. The studied sorghum genotypes responded differently to salinity stress. Thus, the study helps understand the plant tolerance mechanisms of different sweet sorghum genotypes to increasing salinity stress. The study also confirmed that the use of JIP-test is suitable for the identification of sorghum genotypes according to their growth under salinity stress.

Additional key words: abiotic stress; chlorophyll fluorescence; photosynthesis.

Introduction

With an increase of the Earth's population, a significant proportion of grasslands or wetlands are being exploited through intensive cultivation and management (Knox *et al.* 2015, Yamaura *et al.* 2019). The exploitation of different ecosystem leads to damage of the original vegetation and soil structure, which results in soil salinization. The

combination of salinity with drought further adds to the nutritional constraints for agriculture productivity (Kumar *et al.* 2017). Indeed, the development of agriculture, involving systematic use of irrigation, is the main factor behind the extension of this abiotic constraint, which severely limits crop yields and contributes to the degradation of vegetal cover. With the increase in population, the freshwater availability is becoming limited, whereas the

Received 1 November 2019, *accepted* 13 December 2019.

*Corresponding author; phone: 00421-376144448, e-mail: marian.brestic@uniag.sk (M. Brestic); phone: 008618551987196, e-mail: lanygrass@aliyun.com (X. He)

Abbreviations: ABS/CS_m – absorption per excited cross-section; DI₀/CS_m – effective dissipation of energy per excited cross section; ET₀/CS_m – electron transport flux from reduced Q_A to Q_B per excited cross-section; F_0 – minimal intensity of chlorophyll *a* fluorescence, measured when all PSII reaction centers are assumed to be open (at 50 μ s); F_m – maximum fluorescence intensity; F_t – fluorescence intensity at time *t*; F_v/F_m – photosynthetic efficiency of PSII under dark adaptation; LA – leaf area; Lp – plant length; Lr – root length; Ls – shoot length; OEC – oxygen-evolving complex; PI_{ABS} – index of overall photochemical performance; RC/ABS – fraction of active PSII reaction centers; TR₀/CS_m – maximal trapping rate of absorbed photons per excited cross section; V_j – variable fluorescence at time 2 ms; W_k – the amplitude of the K step of OJIP fluorescence transient; ψ_0 – electron transport at PSII acceptor side.

Acknowledgments: This work was supported by the Slovak Research and Development Agency under the project APVV-18-0465, project VEGA-1-0589-19, and National Science Centre Poland under the project UMO-2016/21/B/ST10/02271.

groundwater salinity is increasing (Kabeel and El-Said 2015). Several factors, such as intensive use of saline water in irrigation, evaporation, and condensation due to climate change, low precipitation, *etc.*, play an important role in rapidly increasing saline land throughout the world. Maas and Hoffman (1977) have shown that the crop yield does not get affected until a salinity threshold is exceeded. Research has been performed to show that low salinity does not impact crop productivity, whereas when salt is present in a high concentration, it may severely affect the crop yield (Machado and Serralheiro 2017, Mbarki *et al.* 2018, Arif *et al.* 2019). The mechanism behind the plant response can be related to osmotic stress, ionic toxicity, and ionic imbalances caused by salinity in plants (Kim *et al.* 2007, Munns and Tester 2008). The inhibition of leaf expansion by the salinity was reported to be associated with changes in several factors, such as leaf turgor, net CO₂ assimilation, accumulation of toxic ions, and/or disturbance in mineral nutrition (Bernstein *et al.* 1993). A number of studies have been performed to understand the salinity interaction with photosynthetic machinery in different crops (Sudhir and Murthy 2004, Kalaji *et al.* 2018, Wungrampha *et al.* 2018, Betzen *et al.* 2019).

Among agricultural crops, sorghum is one of the major cereal, which is widely grown in arid, subtropical, semiarid tropical, and temperate regions. In several regions of the world, it is one of the most important sources of food. Sorghum is used as a raw material for brewing and is considered to be a bioenergy crop. As an important crop species, it feeds more than 500 million people in 98 countries (Pennisi 2009). The effects of salt stress and high temperature on sorghum have been widely reported and include the inhibition of photosynthesis and PSII activity (Azhar and McNeilly 1987, 1989; Esehie 1994, Marambe and Ando 1995, Kader and Jutzi 2004, Netondo *et al.* 2004a,b; Prasad *et al.* 2008, Jain *et al.* 2010). A large number of genotypic variation has been reported for the tolerance to salinity in sorghum (Igartua *et al.* 1994, Maiti *et al.* 1994). As the salinity problem is increasing, there is a need for quick identification of salinity tolerant variety in order to improve crop productivity in saline soil.

Photosynthesis is closely related to crop yield but is affected by salinity. The decline of photosynthetic capacity commonly occurred in plants under salt stress, but the underlying mechanisms are complex and not clear (Isayenkov and Maathuis 2019). Chlorophyll (Chl) fluorescence is often used to predict crop yields under various environmental conditions (Baker and Rosenqvist 2004, Kalaji and Pietkiewicz 2004). In recent years, Chl fluorescence parameters were used as the criteria for selecting plants in the breeding programs (Kalaji and Guo 2008, Kalaji *et al.* 2017). It can be used to analyze detailed information about the energy transfer in photosynthesis (Maxwell and Johnson 2000, Kalaji *et al.* 2017). After a dark adaptation, when plants are exposed to actinic light, a rapid increase in Chl fluorescence can be observed and explored to provide information about the status of PSII through a JIP-test (Strasser 1978, 1981). JIP-test uses several mathematical formulae for the purpose to decipher the energy flow in the thylakoids membrane, and is able

to describe primary photosynthetic reactions (Strasser 1978, 1981). In recent years, JIP-test has been successfully applied to understand the mechanism of action of several stress factors in plants (Bussotti *et al.* 2011, Bayat *et al.* 2018, Rastogi *et al.* 2019a). Therefore in this study, we used the JIP-test to understand the salinity tolerance mechanism in sorghum in order to select the best genotype (among studied six genotypes), which can be suitable for growing under salinity conditions.

Materials and methods

Plant material and cultivation conditions: Six sorghum [*Sorghum bicolor* (L.) Moench.] genotypes (five originated from China – Tian Xuan 35, Tian Xuan 90, Tian Xuan 97, Dalishi, and Sutian-1 and one from USA – Rio) were grown under hydroponic cultivation under light-, humidity-, and temperature-controlled conditions. Seeds were surface-disinfected for 30 min using 10% SAVO solution (Biochemie, Bohumín, Czech Republic), which contains 5% NaClO, and washed three times in distilled water. Seeds were germinated on moisture filter paper (Whatman 3) at Petri dishes under 14/10-h day/night regime with light intensity of 150 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ and temperature of 24/18°C. The 7-d-old seedlings were selected for a similar size and transferred to 7-L plastic trays with aerated (regularly six times a day for two hours) Hoagland nutrient solution (pH 6.5). The nutrient solution was changed every four days. Plants were cultivated under 14-h photoperiod, at temperature of 24/18°C, relative humidity of 55–60%, and light intensity of 150 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$. Salt treatment was applied at the stage of 21 d after germination by adding NaCl into Hoagland solution to final concentration 50, 100, 150, 200, and 250 mM, respectively. The time duration of salt treatment was ten days.

Morphological analysis: The length of the aboveground part of a plant (Lp), root (Lr), and shoot (Ls) were measured using caliper on randomly selected plants (7 plants per treatment). Leaf area (LA) was measured for 3rd mature leaf of the plant by drawing the leaf structure on graph paper; further the leaf drawn was cut and measured through leaf area meter CI-202 (CID-Bioscience, USA), with 10 repetitions.

Chl *a* fluorescence: Chl *a* fluorescence transients were measured with Handy-PEA (Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). All fluorescence measurements were taken in the middle part of the 3rd mature leaf (out of the main leaf vein). The leaf samples after 30 min of dark adaptation (using leaf clips) were illuminated with continuous red light (peak in 650 nm wavelength; the spectral line half-width of 22 nm) provided by an array of three light-emitting diodes. The light pulse intensity used was 3,500 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ and the duration of the light pulse was 1 s. The measured data were used for calculation of the JIP-test parameters using BioLyzer v. 3.06 HP software (Strasser *et al.* 2010, Kalaji *et al.* 2016).

Osmotic potential, proline content, and Chl index: Samples of leaves (two circle segments, diameter of 6.0 mm) for measurement of osmotic potential (ψ_s [MPa]) were frozen in liquid nitrogen immediately after the cut and stored at -80°C . Before ψ_s determination, the segment was thawed and inserted into psychrometric chamber C-52 connected to PSYPRO datalogger (Wescor, Logan, Utah, USA). Time interval of temperature and humidity equilibration was set at 60 min and the cooling interval was 15 s.

Free proline content in leaves was determined by the ninhydrin method (Bates *et al.* 1973) using spectrophotometer Jenway 6405 UV/VIS (Jenway, Stone, Staffordshire, UK).

Chl (SPAD) index was measured on the adaxial surface of the 3rd leaf using SPAD-502 chlorophyll meter (Minolta, Japan) immediately after the measurement of Chl *a* fluorescence. The SPAD index values correspond to the amount of Chl present in the leaf and are calculated based on the amount of light transmitted by the leaf in two wavelength regions (650 and 940 nm). SPAD index was an average of ten measurements on the middle part of the leaf (except base and apex).

Sodium and potassium ion concentration: The root and leaves were sampled for dry mass for the purpose to determine Na^+ and K^+ content. Samples were oven-dried at 70°C for 72 h. Na^+ and K^+ content in plant tissues (root and leaves) were determined according to Allen *et al.* (1986) using a flame photometer (FP6410, INESA, China). Dry plant tissues were ground using a mortar and pestle and samples (0.1 g) were digested in 2 ml of sulfuric acid and hydrogen peroxide mixture (3:1). Because the tissue mass of plants grown in few conditions was very low, we pooled the sample together and took only one measurement for the given condition. We selected 150 mM NaCl concentration for the measurement as under this concentration the plants clearly showed the stress response.

Statistical analysis: Each data point represented the mean of seven replicates for morphological and SPAD measurements, four replicates for osmotic potential, and proline analysis, and 3–5 replicates for Chl *a* fluorescence measurements. All data were evaluated by one-way analysis of variance (ANOVA), and the mean differences were compared by post hoc test at *P* level of 0.05. The normal distribution and homogeneity of experimental results were tested by Kolmogorov-Smirnov and Lave's test. In all figures, the spread of values is shown as error bars representing standard error (SE) of the means. All analyses were performed using the *Statistica v. 10* software (StatSoft Inc., Tulsa, Oklahoma, USA).

Results

Morphology of sorghum genotypes: Different sorghum genotypes treated with different concentrations of salt were analyzed for its plant length (Lp), root length (Lr), shoot length (Ls), and the leaf area (LA) (Table 1). The response was dependent on salt concentration in different

genotypes. With NaCl treatment of 250 mM, Tian Xuan 90 showed the least sensitivity; we observed around 5, 44, 12, and 40% decrease in Lp, Lr, Ls, and LA, respectively, whereas cv. Tian Xuan 97 was very sensitive to 250 mM NaCl with around 61, 58, 59, and 76% decrease in Lp, Lr, Ls, and LA, respectively. Interestingly, the salt treatment of 200 mM was observed to be more harmful to Tian Xuan 90 than the impact of 250 mM salt concentration, especially for plant length. LA was the least in genotype Rio under 250 mM concentration. The shortest root was observed in Tian Xuan 97 under 250 mM salt treatment.

Transient fluorescence curves: Transient fluorescence curves, also known as OJIP curves, were analyzed for six sorghum genotypes exposed to different concentrations of salt (0, 100, 150, 200, and 250 mM). For better visualization, the curve was normalized for F_0 (fluorescence at time 0) and presented as F_t/F_0 (where F_t is fluorescence at time *t*) against the time (Fig. 1). The figure shows the variable response to salinity stress on OJIP curves for different sorghum genotypes. Apart from O, J, I, and P steps in the OJIP curve, some other peaks may appear, which indicates various phenomena of photosynthetic mechanism (Kalaji *et al.* 2014). Among them, K step is an important step which indicates a decrease in oxygen-evolving complex (OEC) capacity due to the impairment of electron transport between the OEC and reaction center of PSII (Srivastava *et al.* 1997). Generally, these additional effects were masked under moderate stress conditions in the graph with absolute value of Chl *a* fluorescence transient curve. Therefore, the mathematically double-normalized values of OJ phase, *i.e.*, the time plots of relative variable fluorescence V_t/V_j from 0.05 to 2 ms, were used in the analysis (Fig. 2). The graph indicates a different response of salinity stress at K step for sorghum genotypes.

Different photosynthetic parameters: Among various photosynthetic parameters, maximal photochemical efficiency of PSII (F_v/F_m) and performance index (PI_{ABS}) were selected to be represented in this manuscript due to its proven sensitivity to identify the abiotic stress response in different plants (Kalaji *et al.* 2017, Rastogi *et al.* 2019a,b). F_v/F_m indicates the maximum quantum yield of PSII photochemistry, whereas PI_{ABS} is a photosynthetic parameter that takes into consideration a number of different phenomena related to PSII photochemistry (Zivcak *et al.* 2008). With an increase in the salt concentration, the impact of salinity on different sorghum genotypes was different (Fig. 3). Other important photosynthetic parameters obtained through JIP-test analysis, which represent the activity of acceptor and donor sites of PSII, were analyzed and presented in Fig. 4. Relative variable fluorescence at 2 ms (V_i), the probability that a trapped exciton transfers as electron into the electron transport chain beyond Q_A (ψ_0), the ratio of the K phase to the J phase (W_k) and the fraction of OEC in relation to control plants were observed and presented for its differences in different sorghum genotypes under different salinity conditions.

Table 1. Morphological characterization of sorghum genotypes treated by different concentrations of NaCl in hydroponic solution. Lp – plant length, Lr – root length, Ls – shoot length, LA – leaf area. The data represent means $\pm$ SE, $n = 7$. *Uppercase letters and lowercase letters* denote significant difference (*Duncan's* post hoc test) between genotypes at a given growth NaCl concentration level and all treatments for given sorghum genotype at $p < 0.05$, respectively.

Genotype	NaCl [mM]	Lp [mm]	Lr [mm]	Ls [mm]	LA [cm ²]
Tian Xuan 35	0	243.6 $\pm$ 19.7 ^{Ba}	249.4 $\pm$ 15.0 ^{Aa}	59.2 $\pm$ 5.1 ^{Ba}	29.4 $\pm$ 3.2 ^{Ba}
	100	235.0 $\pm$ 23.4 ^{Aa}	175.7 $\pm$ 3.8 ^{Aa}	54.0 $\pm$ 5.3 ^{Ba}	28.2 $\pm$ 5.5 ^{Aa}
	150	178.3 $\pm$ 15.0 ^{Bb}	228.0 $\pm$ 28.8 ^{Ab}	52.7 $\pm$ 7.1 ^{Ba}	15.4 $\pm$ 4.7 ^{Bb}
	200	201.7 $\pm$ 14.5 ^{Ab}	162.7 $\pm$ 10.5 ^{Ab}	59.0 $\pm$ 3.0 ^{Ba}	18.2 $\pm$ 2.6 ^{Bb}
	250	200.0 $\pm$ 19.8 ^{ABb}	178.5 $\pm$ 6.4 ^{Ab}	54.5 $\pm$ 2.1 ^{Ba}	15.7 $\pm$ 3.6 ^{ABb}
Tian Xuan 90	0	188.0 $\pm$ 8.0 ^{CDa}	215.4 $\pm$ 31.0 ^{ABCa}	57.0 $\pm$ 3.9 ^{Bab}	18.0 $\pm$ 2.5 ^{CDa}
	100	176.0 $\pm$ 32.8 ^{Bab}	164.0 $\pm$ 10.1 ^{Ab}	57.7 $\pm$ 6.1 ^{ABa}	17.3 $\pm$ 6.5 ^{Ba}
	150	175.7 $\pm$ 20.2 ^{Bab}	170.0 $\pm$ 36.3 ^{BCb}	52.3 $\pm$ 3.1 ^{Bab}	8.6 $\pm$ 3.0 ^{BCb}
	200	149.7 $\pm$ 23.5 ^{Bb}	161.7 $\pm$ 24.4 ^{Ab}	52.7 $\pm$ 2.5 ^{Bab}	11.2 $\pm$ 1.4 ^{Cab}
	250	179.7 $\pm$ 14.8 ^{Bab}	144.3 $\pm$ 10.8 ^{Bb}	50.0 $\pm$ 2.6 ^{Bb}	10.9 $\pm$ 1.1 ^{BCab}
Tian Xuan 97	0	159.6 $\pm$ 34.8 ^{Da}	185.8 $\pm$ 16.7 ^{Ca}	52.4 $\pm$ 8.4 ^{Ba}	11.2 $\pm$ 5.6 ^{Dab}
	100	173.3 $\pm$ 22.5 ^{Ba}	163.0 $\pm$ 11.0 ^{Aa}	51.3 $\pm$ 7.8 ^{Ba}	13.8 $\pm$ 4.4 ^{Ba}
	150	150.3 $\pm$ 10.6 ^{Bab}	127.7 $\pm$ 12.7 ^{Cb}	45.7 $\pm$ 8.5 ^{Ba}	7.4 $\pm$ 4.7 ^{Cabc}
	200	106.7 $\pm$ 13.9 ^{Bbc}	100.7 $\pm$ 27.3 ^{Cbc}	31.3 $\pm$ 1.2 ^{Cb}	4.2 $\pm$ 1.6 ^{Dbc}
	250	62.0 $\pm$ 7.0 ^{Dc}	79.3 $\pm$ 18.5 ^{Dc}	21.7 $\pm$ 1.5 ^{Eb}	2.7 $\pm$ 1.1 ^{Dc}
Dalishi	0	293.0 $\pm$ 39.0 ^{Aa}	201.0 $\pm$ 36.5 ^{BCa}	81.3 $\pm$ 4.6 ^{Aa}	54.7 $\pm$ 7.7 ^{Aa}
	100	197.3 $\pm$ 9.9 ^{Bb}	159.3 $\pm$ 15.2 ^{Ab}	66.3 $\pm$ 3.1 ^{Ab}	33.4 $\pm$ 4.4 ^{Ab}
	150	232.3 $\pm$ 21.7 ^{Ab}	184.3 $\pm$ 17.5 ^{ABb}	68.0 $\pm$ 6.6 ^{Ab}	30.4 $\pm$ 1.7 ^{Ab}
	200	241.3 $\pm$ 15.9 ^{Ab}	148.0 $\pm$ 8.9 ^{ABb}	69.3 $\pm$ 6.5 ^{Ab}	31.8 $\pm$ 4.6 ^{Ab}
	250	219.0 $\pm$ 21.3 ^{Ab}	153.3 $\pm$ 12.7 ^{Bb}	60.3 $\pm$ 4.5 ^{Ab}	21.1 $\pm$ 5.3 ^{Ac}
Rio	0	212.6 $\pm$ 13.3 ^{BCa}	237.0 $\pm$ 14.2 ^{ABa}	58.7 $\pm$ 3.5 ^{Ba}	21.8 $\pm$ 2.9 ^{Ca}
	100	186.3 $\pm$ 8.4 ^{Bab}	176.0 $\pm$ 4.2 ^{Ab}	52.7 $\pm$ 2.1 ^{Bb}	17.0 $\pm$ 2.5 ^{Bab}
	150	167.3 $\pm$ 8.0 ^{Bb}	156.3 $\pm$ 13.8 ^{BCb}	49.0 $\pm$ 4.4 ^{Bb}	14.1 $\pm$ 3.4 ^{BCbc}
	200	128.7 $\pm$ 28.4 ^{Bc}	112.7 $\pm$ 26.6 ^{BCc}	35.0 $\pm$ 2.8 ^{Cc}	9.1 $\pm$ 3.6 ^{CDcd}
	250	129.7 $\pm$ 3.2 ^{Cc}	103.3 $\pm$ 7.6 ^{Cc}	29.0 $\pm$ 1.0 ^{Dd}	5.6 $\pm$ 1.0 ^{CDd}
Sutian-1	0	181.0 $\pm$ 16.7 ^{CDb}	121.3 $\pm$ 7.0 ^{Da}	60.7 $\pm$ 1.5 ^{Bb}	15.9 $\pm$ 2.6 ^{CDb}
	100	236.7 $\pm$ 15.7 ^{Ba}	151.0 $\pm$ 10.8 ^{Ba}	71.3 $\pm$ 5.7 ^{ABa}	26.6 $\pm$ 6.7 ^{Ba}
	150	163.0 $\pm$ 27.9 ^{Bbc}	124.0 $\pm$ 31.2 ^{Ca}	49.0 $\pm$ 10.6 ^{Bc}	13.1 $\pm$ 4.1 ^{BCbc}
	200	137.3 $\pm$ 15.0 ^{Bc}	125.3 $\pm$ 17.0 ^{ABCa}	32.3 $\pm$ 2.5 ^{Cd}	8.4 $\pm$ 3.1 ^{CDbc}
	250	143.3 $\pm$ 7.1 ^{Cc}	87.0 $\pm$ 4.6 ^{CDb}	39.7 $\pm$ 3.1 ^{Ccd}	7.2 $\pm$ 1.2 ^{CDc}

Leaf model for energy flux: For a better understanding of photosystem functioning, the *Biolyzer* software-generated leaf model was presented in Fig. 5. The figure shows the proportion of phenomenological energy fluxes within a leaf, calculated based on per excited cross section (CS_m).

Osmotic potential, proline concentration, and Chl concentration: Salt is known to interfere with solute movement, therefore, the osmotic potential of plants was measured and observed to be increasing with the increase in salt concentration (Table 2). Proline is the amino acid, which is elevated under stress conditions (Hayat *et al.* 2012); in this study, the concentration of proline increased with the increase in salt concentrations for different sorghum genotypes (Table 2). Chl concentration is the factor that is generally decreasing under plant stress conditions; the SPAD index indicated a decrease in Chl concentration due to salinity in studied sorghum genotypes.

Na⁺ and K⁺ concentration: The ratio of Na⁺ and K⁺ increased significantly in salt-treated samples (Table 3) for studied sorghum genotypes, which was due to a significant increase in Na⁺ due to the salt treatment, which also replaced K⁺; as a result, the concentration of K⁺ decreased in the treated samples.

Discussion

Different stress factors interact differently with different plants, but we start recognizing it only when we see their impacts on plant growth parameters, such as plant size, leaf shape, yield, *etc.* Therefore, for this study, we first focused on morphological parameters and observed a significant decrease in plant length, root length, shoot length, and leaf area with an increasing concentration of salt. Morphological variation showed that Tian Xuan 97 was the most sensitive genotype to salt stress, whereas Tian Xuan 90 was the least

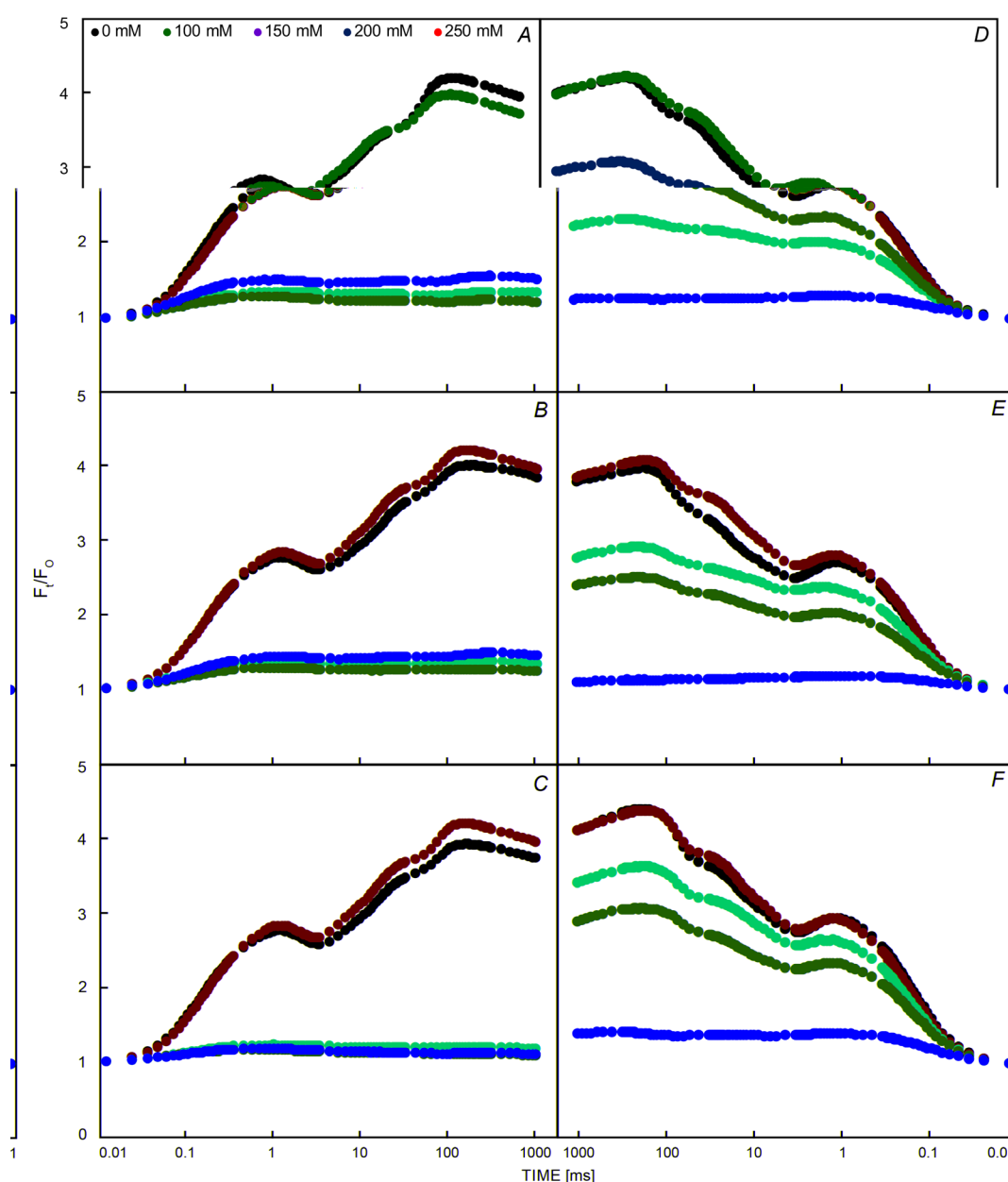


Fig. 1. Chlorophyll *a* normalized fluorescence intensity (F_t) relative to F_0 (A–F) in sorghum leaves of different genotypes affected by different NaCl concentration. Genotypes: Tian Xuan 35 (A), Tian Xuan 90 (B), Tian Xuan 97 (C), Dalishi (D), Rio (E), and Sutian-1 (F). Data represent the mean ($n = 3–5$).

sensitive genotype of sorghum. But the morphological changes are not sufficient to indicate why the deviation in growth parameters happened, and the morphological deviations are identified mostly at a later stage of the plant life cycle. Therefore, different methods have been developed for the purpose to identify the stress response at an early stage. Some of the methods are destructive and some are noninvasive. The noninvasive and quick methods are the most useful for farmers as they can avoid the loss of the crop yield by early detection of the stress factor. Recently, the JIP-test has established itself as a quick and reliable technique for field identification of different stress

factors at the early stage (Bussotti *et al.* 2011, Kalaji *et al.* 2014, Rastogi *et al.* 2019a). Chl induction curve is an indicator of different phenomena of photosynthetic light reactions. Significant suppression in Chl induction curve clearly indicated that Tian Xuan 35, Tian Xuan 90, and Tian Xuan 97 are salt-sensitive genotypes, whereas Dalishi, Rio, and Sutian-1 are relatively salt-resistant sorghum genotypes, where photosynthetic machinery was functioning even at salt concentration of 200 mM. The studied genotypes of sorghum were resistant to 100 mM salt except Tian Xuan 97, which was highly sensitive to salt stress. The sensitivity of Tian Xuan 97 was clear with a

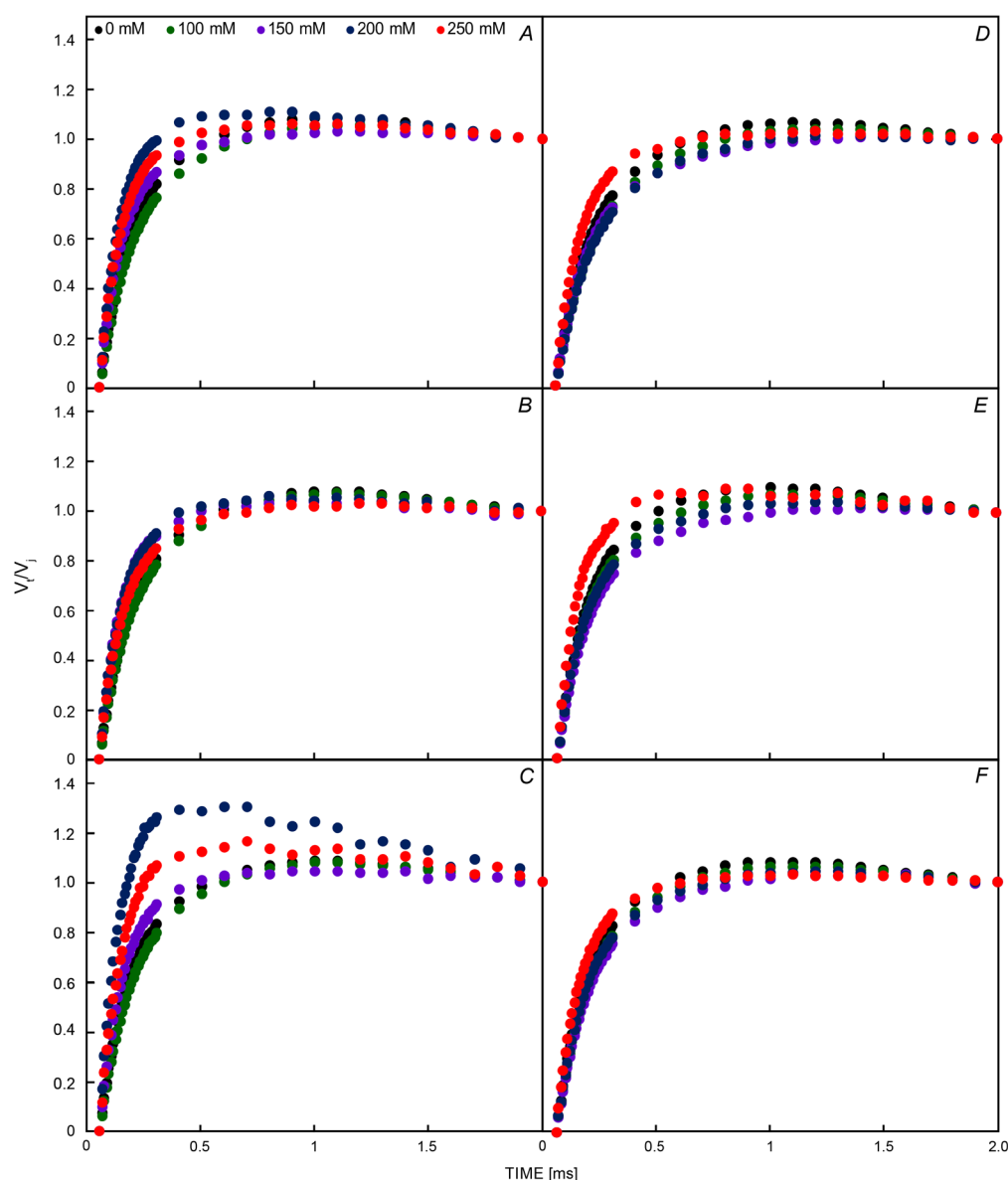


Fig. 2. Chlorophyll *a* variable fluorescence (V_t) relative to variable fluorescence at 2 ms (V_j) (A–F) in sorghum leaves of different genotypes affected by different NaCl concentration. Genotypes: Tian Xuan 35 (A), Tian Xuan 90 (B), Tian Xuan 97 (C), Dalishi (D), Rio (E), and Sutian-1 (F). Data represent the mean ($n = 3-5$).

clear deviation observed for K step, indicating dissociation of the OEC (Oukarroum *et al.* 2007), thus showing significant damage to the photosystems. The observation of photosynthetic parameters, such as F_v/F_m and PI_{ABS} , clearly indicated the lowest value for Tian Xuan 97, which further confirmed the claim that Tian Xuan 97 was the most sensitive genotype. Data also indicated that the plants were able to tolerate the salt concentration up to 100 mM, but 150 mM was already lethal for salt-sensitive varieties, *i.e.*, Tian Xuan 35, Tian Xuan 90, and Tian Xuan 97. As the salt-resistant varieties also showed a significant reduction in F_v/F_m and PI_{ABS} at 150 mM, this concentration was identified as the threshold level, harmful to all studied genotypes. The best photosynthetically performing geno-

type was Sutain-1 which showed a significantly better Chl induction curve, F_v/F_m , and PI_{ABS} when compared to other salt-resistant genotypes. The same was visible in other photosynthetic parameters studied in Fig. 4, where a number of closed reaction centers was higher for sensitive varieties as seen by V_j . The probability of electron to pass through Q_A^- was also low for sensitive genotypes, in the form of lower value of ψ_0 (Kalaji *et al.* 2017). The ratio of K phase to the J phase (W_k) was high for Tian Xuan 97. The value of W_k indicates the activity of the OECs on the donor side of PSII where an increase in W_k indicates damage to the complexes. The observation was confirmed by the values of the fraction of active oxygen-evolving complex which fell down to zero at 150 mM in Tian Xuan

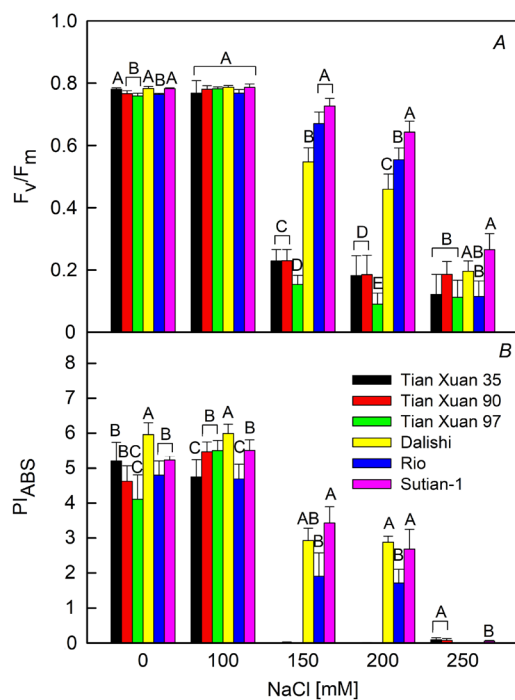


Fig. 3. Maximal photochemical efficiency of PSII (F_v/F_m) and performance index (PI_{ABS}) in sorghum genotypes affected by different NaCl concentration in hydroponic solution. Data represent the mean $\pm$ SE ($n = 3-5$). Different letters indicate the significant differences between genotypes (Duncan's post hoc test, $p < 0.05$).

97 genotype, whereas, the fraction of active OEC was high up to 200 mM in the resistant genotypes. In the leaf pipeline model presented, we can see that the number of inactive reaction centers was high (shown by dark cycle) for plants treated with 150 mM and higher salt concentration except for genotype Sutian-1 (which was the most salt-tolerant one). Similar to a number of reaction centers, the light-use efficiency of different plants also decreased which can be seen by smaller sizes of ABS/CS_m and TR_0/CS_m , where ABS/CS_m indicates light absorbed by per cross section and TR_0/CS_m indicates trapped light energy per cross section. Electron flux per cross section of leaf (ET_0/CS_m) was negligible for salt-sensitive genotypes, whereas it was significantly higher for the salt-resistant genotypes.

To see further the impact of salinity on some other physiological processes, we measured osmotic potential (ψ_s), proline content (Pro), and SPAD index. Different studies have shown that the accumulation of salts in the root zone may cause a decrease in ψ_s (Franco *et al.* 2011), which was lower with the increase of NaCl concentration in different sorghum genotypes in our study. Tian Xuan 70, being the most sensitive, did not show any measurable ψ_s for 150, 200, and 250 mM NaCl. An overproduction of proline is a common phenomenon in plants under stress. The increase in the proline concentration has a positive effects on stress tolerance by maintaining cell turgor, preventing electrolyte leakage, and preventing plants from oxidative burst (Hayat *et al.* 2012). The increase in the proline content under salinity stress was observed in different sorghum genotypes, which is in agreement

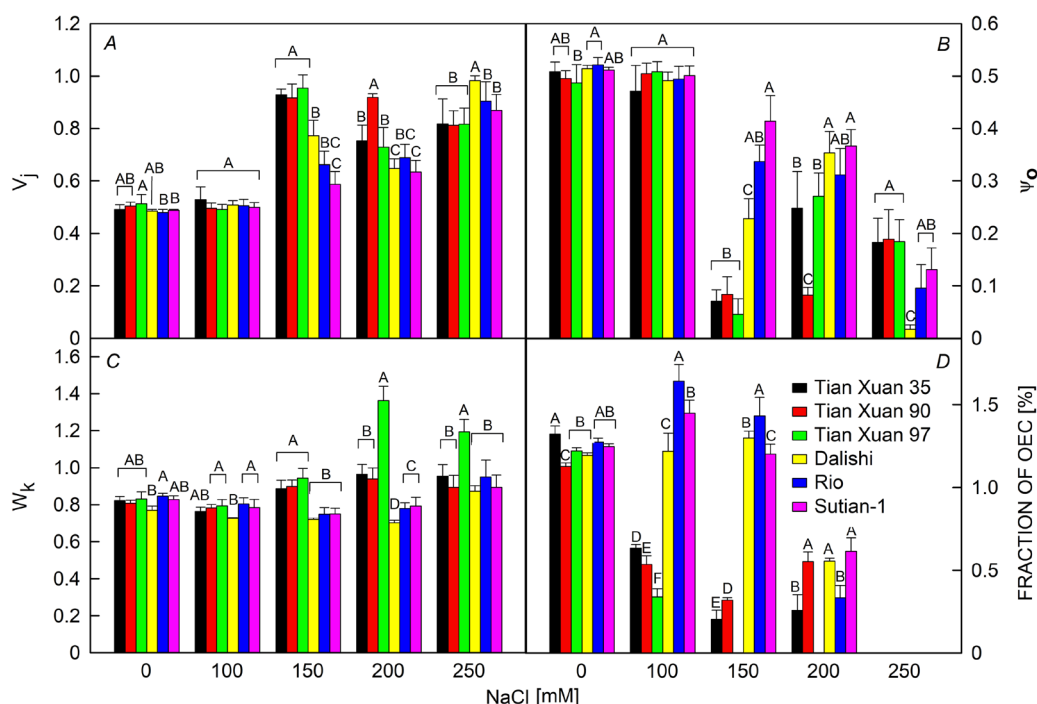


Fig. 4. Parameters reflected the activity of acceptor (A,B) and donor (C,D) side of PSII in sorghum leaves affected by different NaCl concentration. (A) Relative variable fluorescence at 2 ms (V_j). (B) Probability that a trapped exciton transfers as electron into the electron transport chain beyond Q_A (ψ_0). (C) Ratio of the K phase to the J phase (W_k). (D) The fraction of oxygen-evolving complex relative to control plants. Data represent the mean $\pm$ SE ($n = 3-5$). Different letters indicate the significant differences between genotypes (Duncan's post hoc test, $p < 0.05$).

Table 2. Osmotic potential (ψ_s), proline content (Pro), and SPAD index in sorghum genotypes treated by different concentrations of NaCl in hydroponic solution. The data represent means $\pm$ SE, $n = 7$ for SPAD and $n = 4$ for ψ_s and Pro, respectively. n.m. – not measurable, FM – fresh mass. *Uppercase letters and lowercase letters* denote significant differences (*Duncan's* post hoc test) between genotypes at different NaCl concentration levels ($p < 0.05$), respectively.

Genotype	NaCl [mM]	ψ_s [MPa]	Pro [$\mu\text{mol g}^{-1}$ (FM)]	SPAD index
Tian Xuan 35	0	$-1.28 \pm 0.25^{\text{Aa}}$	$0.60 \pm 0.02^{\text{Ba}}$	$35.5 \pm 1.6^{\text{Ba}}$
	100	$-1.11 \pm 0.23^{\text{Aa}}$	$0.99 \pm 0.07^{\text{Ca}}$	$33.4 \pm 1.3^{\text{ABa}}$
	150	$-3.69 \pm 0.29^{\text{Cb}}$	$52.27 \pm 7.80^{\text{Cb}}$	$25.9 \pm 0.8^{\text{ABb}}$
	200	$-3.84 \pm 0.62^{\text{Ab}}$	$54.23 \pm 4.36^{\text{Ab}}$	$24.0 \pm 1.7^{\text{Bbc}}$
	250	n.m.	$65.50 \pm 3.15^{\text{Ba}}$	$24.2 \pm 1.3^{\text{Ac}}$
Tian Xuan 90	0	$-1.42 \pm 0.51^{\text{Aa}}$	$0.92 \pm 0.53^{\text{Bc}}$	$35.5 \pm 1.6^{\text{Ba}}$
	100	$-1.35 \pm 0.06^{\text{Aa}}$	$1.34 \pm 0.25^{\text{Cc}}$	$35.6 \pm 0.9^{\text{Aa}}$
	150	$-2.00 \pm 0.48^{\text{Aa}}$	$73.47 \pm 12.98^{\text{Ab}}$	$24.2 \pm 2.8^{\text{BCb}}$
	200	n.m.	n.m.	$24.0 \pm 0.7^{\text{Bb}}$
	250	n.m.	$143.19 \pm 15.21^{\text{Aa}}$	$24.8 \pm 1.2^{\text{Ab}}$
Tian Xuan 97	0	$-1.26 \pm 0.13^{\text{Aa}}$	$2.39 \pm 0.51^{\text{Ac}}$	$32.6 \pm 0.9^{\text{Ca}}$
	100	$-1.28 \pm 0.07^{\text{Aa}}$	$6.73 \pm 2.00^{\text{Bc}}$	$32.6 \pm 1.3^{\text{Ca}}$
	150	n.m.	$48.92 \pm 3.41^{\text{Cb}}$	$23.3 \pm 1.2^{\text{Cb}}$
	200	n.m.	n.m.	$23.3 \pm 2.2^{\text{Bb}}$
	250	n.m.	$89.09 \pm 7.58^{\text{Ba}}$	$21.3 \pm 2.8^{\text{Ab}}$
Dalishi	0	$-0.96 \pm 0.27^{\text{Aa}}$	$0.73 \pm 0.16^{\text{Bc}}$	$38.6 \pm 1.7^{\text{Aa}}$
	100	$-1.76 \pm 0.19^{\text{Bb}}$	$9.69 \pm 3.02^{\text{Bb}}$	$32.7 \pm 1.4^{\text{Cb}}$
	150	$-2.41 \pm 0.37^{\text{ABc}}$	$25.88 \pm 3.37^{\text{Da}}$	$26.8 \pm 1.4^{\text{Acd}}$
	200	$-2.73 \pm 0.41^{\text{Ac}}$	$29.78 \pm 4.47^{\text{Ba}}$	$26.9 \pm 2.6^{\text{ABcd}}$
	250	$-4.65 \pm 0.36^{\text{d}}$	n.m.	$24.0 \pm 3.2^{\text{Ad}}$
Rio	0	$-0.98 \pm 0.23^{\text{Aa}}$	$1.23 \pm 0.27^{\text{Bc}}$	$35.7 \pm 1.1^{\text{Ba}}$
	100	$-2.23 \pm 0.12^{\text{Cb}}$	$16.57 \pm 2.34^{\text{Ad}}$	$33.3 \pm 3.2^{\text{ABa}}$
	150	$-2.07 \pm 0.39^{\text{Ab}}$	$32.71 \pm 6.68^{\text{Dc}}$	$26.2 \pm 0.9^{\text{ABb}}$
	200	$-3.52 \pm 0.41^{\text{Ac}}$	$57.16 \pm 12.84^{\text{Ab}}$	$26.2 \pm 3.8^{\text{ABb}}$
	250	n.m.	$79.42 \pm 7.34^{\text{Ba}}$	$24.4 \pm 3.3^{\text{Ab}}$
Sutian-1	0	$-1.03 \pm 0.17^{\text{Aa}}$	$1.05 \pm 0.21^{\text{Bd}}$	$34.1 \pm 2.3^{\text{BCa}}$
	100	$-2.11 \pm 0.12^{\text{Cb}}$	$1.41 \pm 0.28^{\text{Cd}}$	$32.6 \pm 2.0^{\text{Ca}}$
	150	$-2.77 \pm 0.18^{\text{Bb}}$	$10.25 \pm 1.99^{\text{Bc}}$	$26.1 \pm 1.1^{\text{ABb}}$
	200	$-2.87 \pm 0.72^{\text{Ac}}$	$25.62 \pm 6.91^{\text{Bb}}$	$27.8 \pm 2.9^{\text{Ab}}$
	250	n.m.	$78.57 \pm 14.85^{\text{Ba}}$	$22.4 \pm 1.6^{\text{Ac}}$

Table 3. Accumulation of Na^+ and K^+ in sorghum genotypes treated by different concentration of NaCl in hydroponic solution.

Genotype	NaCl [mM]	Root content	Leaves content	Na^+/K^+	Na^+ [mg g^{-1}]	K^+ [mg g^{-1}]	Na^+/K^+
		Na^+ [mg g^{-1}]	K^+ [mg g^{-1}]				
Tian Xuan 35	0	5.94	38.40	0.15	5.57	44.96	0.12
	150	22.86	36.52	0.63	36.57	26.26	1.39
Tian Xuan 90	0	3.01	30.22	0.10	2.79	27.59	0.10
	150	26.99	21.69	1.24	39.94	24.90	1.60
Tian Xuan 97	0	9.05	28.11	0.32	9.75	24.71	0.39
	150	38.84	22.20	1.75	43.94	32.40	1.36
Dalishi	0	1.51	45.92	0.03	0.49	49.82	0.01
	150	33.04	32.11	1.03	27.56	24.93	1.11
Rio	0	6.43	41.41	0.16	2.83	28.49	0.10
	150	23.05	20.73	1.11	27.93	30.82	0.91
Sutian-1	0	2.28	46.29	0.05	8.43	24.24	0.35
	150	28.60	33.15	0.86	37.62	27.77	1.35

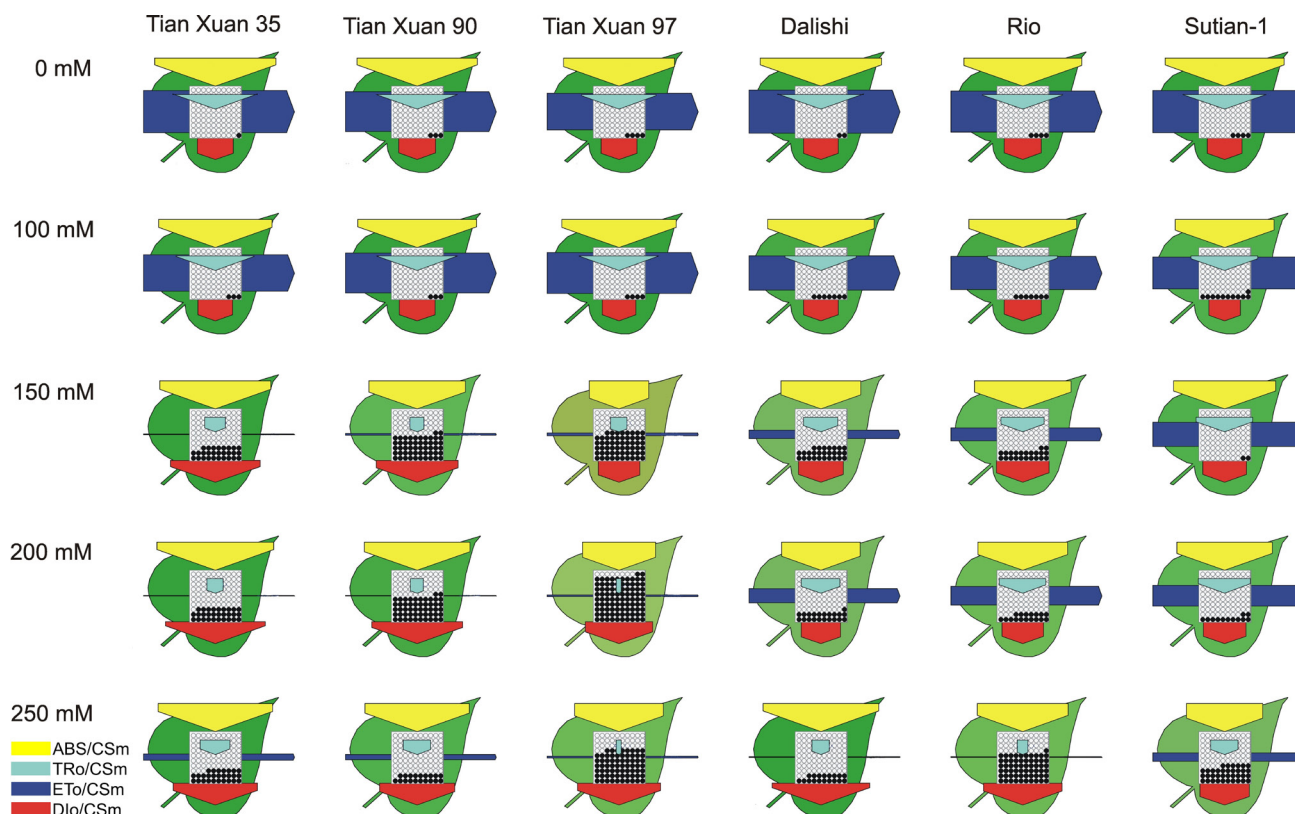


Fig. 5. Leaf pipeline model showing the proportion of phenomenological energy fluxes within a leaf calculated per excited cross section (CS_m). Models are drawn with *Biolzyzer* software. The value of each parameter can be seen in relative changes in width of each arrow (see the color legend). Active RCs are shown as *open circles* and inactive RCs are *closed circles*. ABS/CS_m – absorption per excited cross section; DI_o/CS_m – effective dissipation of energy per excited cross section; ET_o/CS_m – electron transport flux from reduced Q_A to Q_B per excited cross section; TR_o/CS_m – maximal trapping rate of absorbed photons per excited cross section.

with previous studies on sorghum and other plants (Al-Karaki *et al.* 1996, Iqbal *et al.* 2014, Hmidi *et al.* 2018). Interestingly, Tian Xuan 97 at 250 mM NaCl showed the maximum amount of proline, which might indicate the hyperactive protective mechanism of the plant under such a high salt concentration. SPAD index varied slightly for different genotypes under the same salt concentration indicating its lesser sensitivity for the identification of salt stress, while the variation in proline and osmotic potential were significant between genotypes, which indicated that these parameters are more reliable for the given purpose, *i.e.*, to assess the salt stress in comparison to SPAD index.

The concentration of Na^+ and K^+ ion was measured for 150 mM salt concentration; Na^+ concentration was higher in leaves of salt-sensitive genotypes, whereas comparatively lower in leaves of salt-resistant genotypes. The concentration of Na^+ was similar in roots of salt-resistant and salt-sensitive sorghum genotypes, indicating that the transportation of Na^+ in salt-resistant genotypes was significantly reduced, thus indicating the resistant mechanism for salt-resistant genotypes.

The study clearly indicated how JIP-test can be used to identify the salt-resistant genotypes of sorghum, with an indication of the sensitive photosynthetic parameters.

Compared to chlorophyll fluorescence, the chlorophyll concentration was found to be a less sensitive parameter for identifying the susceptible individuals. In addition, our results suggest that the regulation of ion transport may have an important role for salt resistance in sorghum genotypes.

References

- Al-Karaki G.N., Al-Karaki R.B., Al-Karaki C.Y.: Phosphorus nutrition and water stress effects on proline accumulation in sorghum and bean. – *J. Plant Physiol.* **148**: 745-751, 1996.
- Allen S.E., Grimshaw H.M., Rowland A.P.: Chemical analysis. – In: Moore P.D., Chapman S.B. (ed.): *Methods in Plant Ecology*. Pp. 285-344. Blackwell Scientific Publications, Oxford 1986.
- Arif M.R., Islam M.T., Robin A.H.K.: Salinity stress alters root morphology and root hair traits in *Brassica napus*. – *Plants* **8**: 192, 2019.
- Azhar F.M., McNeilly T.: Variability for salt tolerance in *Sorghum bicolor* (L.) Moench under hydroponic condition. – *J. Agron. Crop Sci.* **159**: 269-277, 1987.
- Azhar F.M., McNeilly T.: The response of four sorghum accessions/cultivars to salinity during plant development. – *J. Agron. Crop Sci.* **161**: 33-43, 1989.

- Baker N.R., Rosenqvist E.: Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. – *J. Exp. Bot.* **55**: 1607-1621, 2004.
- Bates L., Waldren R.P., Teare I.D.: Rapid determination of free proline for water-stress studies. – *Plant Soil* **39**: 205-207, 1973.
- Bayat L., Arab M., Aliniaefard S. *et al.*: Effects of growth under different light spectra on the subsequent high light tolerance in rose plants. – *AoB Plants* **10**: ply052, 2018.
- Bernstein N., Läuchli A., Silk W.K.: Kinematics and dynamics of sorghum (*Sorghum bicolor* L.) leaf development at various Na/Ca salinities: I. Elongation growth. – *Plant Physiol.* **103**: 1107-1114, 1993.
- Betzen B.M., Smart C.M., Maricle K.L., Maricle B.R.: Effects of increasing salinity on photosynthesis and plant water potential in kansas salt marsh species. – *Trans. Kans. Acad. Sci.* **122**: 29-58, 2019.
- Bussotti F., Desotgiu R., Cascio C. *et al.*: Ozone stress in woody plants assessed with chlorophyll *a* fluorescence. A critical reassessment of existing data. – *Environ. Exp. Bot.* **73**: 19-30, 2011.
- Esechie H.A.: Interaction of salinity and temperature on the germination of sorghum. – *J. Agron. Crop Sci.* **172**: 194-199, 1994.
- Franco J.A., Bañón S., Vicente M.J. *et al.*: Root development in horticultural plants grown under abiotic stress conditions – a review. – *J. Hortic. Sci. Biotech.* **86**: 543-556, 2011.
- Hayat S., Hayat Q., Alyemeni M.N. *et al.*: Role of proline under changing environments: a review. – *Plant Signal. Behav.* **7**: 1456-1466, 2012.
- Hmidi D., Abdelly C., Athar H.U. *et al.*: Effect of salinity on osmotic adjustment, proline accumulation and possible role of ornithine- δ -aminotransferase in proline biosynthesis in *Cakile maritima*. – *Physiol. Mol. Biol. Pla.* **24**: 1017-1033, 2018.
- Igartua E., Gracia M.P., Lasa J.M.: Characterization and genetic control of germination-emergence responses of grain sorghum to salinity. – *Euphytica* **76**: 185-193, 1994.
- Iqbal N., Umar S., Khan N.A., Khan M.I.R.: A new perspective of phytohormones in salinity tolerance: Regulation of proline metabolism. – *Environ. Exp. Bot.* **100**: 34-42, 2014.
- Isayenkov S.V., Maathuis F.J.M.: Plant salinity stress: Many unanswered questions remain. – *Front. Plant Sci.* **10**: 80, 2019.
- Jain M., Chourey P.S., Boote K.J., Allen Jr. L.H.: Short-term high temperature growth conditions during vegetative-to-reproductive phase transition irreversibly compromise cell wall invertase-mediated sucrose catalysis and microspore meiosis in grain sorghum (*Sorghum bicolor*). – *J. Plant Physiol.* **167**: 578-582, 2010.
- Kabeel A.E., El-Said E.M.S.: Water production for irrigation and drinking needs in remote arid communities using closed-system greenhouse: A review. – *Eng. Sci. Technol. Int. J.* **18**: 294-301, 2015.
- Kader M.A., Jutzi S.C.: Effects of thermal and salt treatments during imbibition on germination and seedling growth of sorghum at 42/19°C. – *J. Agron. Crop Sci.* **190**: 35-38, 2004.
- Kalaji H.M., Goltsev V.N., Żuk-Golaszewska K. *et al.*: Chlorophyll Fluorescence: Understanding Crop Performance – Basics and Applications. Pp. 222. CRC Press, Boca Raton 2017.
- Kalaji H.M., Guo P.: Chlorophyll fluorescence: a useful tool in barley plant breeding programs. – In: Sánchez A., Gutiérrez S.J. (ed.): *Photochemistry Research Progress*. Pp. 439-463. Nova Science Publishers, Inc., New York 2008.
- Kalaji H.M., Jajoo A., Oukarroum A. *et al.*: Chlorophyll *a* fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. – *Acta Physiol. Plant.* **38**: 102, 2016.
- Kalaji H.M., Pietkiewicz S.: Some physiological indices to be exploited as a crucial tool in plant breeding. – *Plant Breed. Seed Sci.* **49**: 19-39, 2004.
- Kalaji H.M., Rastogi A., Zivcak M. *et al.*: Prompt chlorophyll fluorescence as a tool for crop phenotyping: an example of barley landraces exposed to various abiotic stress factors. – *Photosynthetica* **56**: 953-961, 2018.
- Kalaji H.M., Schansker G., Ladle R.J. *et al.*: Frequently asked questions about *in vivo* chlorophyll fluorescence: practical issues. – *Photosynth. Res.* **122**: 121-158, 2014.
- Kim D.-W., Shibato J., Agrawal G.K. *et al.*: Gene transcription in the leaves of rice undergoing salt-induced morphological changes (*Oryza sativa* L.). – *Mol. Cells* **24**: 45-59, 2007.
- Knox S.H., Sturtevant C., Matthes J.H. *et al.*: Agricultural peatland restoration: Effects of land-use change on greenhouse gas (CO₂ and CH₄) fluxes in the Sacramento-San Joaquin Delta. – *Glob. Change Biol.* **21**: 750-764, 2015.
- Kumar D., Al Hassan M., Naranjo M.A. *et al.*: Effects of salinity and drought on growth, ionic relations, compatible solutes and activation of antioxidant systems in oleander (*Nerium oleander* L.). – *PLoS ONE* **12**: e0185017, 2017.
- Maas E.V., Hoffman G.J.: Crop salt tolerance – current assessment. – *J. Irr. Drain. Div.-ASCE* **103**: 115-134, 1977.
- Machado R.M.A., Serralheiro R.P.: Soil salinity: Effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. – *Horticulturae* **3**: 30, 2017.
- Maiti R.K., de la Rosa-Ibarra M., Sandoval N.D.: Genotypic variability in glossy sorghum lines for resistance to drought, salinity and temperature stress at the seedling stage. – *J. Plant Physiol.* **143**: 241-244, 1994.
- Marambe B., Ando T.: Physiological basis of salinity tolerance of sorghum seeds during germination. – *J. Agron. Crop Sci.* **174**: 291-296, 1995.
- Maxwell K., Johnson G.N.: Chlorophyll fluorescence – a practical guide. – *J. Exp. Bot.* **51**: 659-668, 2000.
- Mbarki S., Sytar O., Cerda A. *et al.*: Strategies to mitigate the salt stress effects on photosynthetic apparatus and productivity of crop plants. – In: Kumar V., Wani S.H., Suprasanna P., Tran L.S.P. (ed.): *Salinity Responses and Tolerance in Plants*. Pp. 85-136. Springer, Cham 2018.
- Munns R., Tester M.: Mechanisms of salinity tolerance. – *Annu. Rev. Plant Biol.* **59**: 651-681, 2008.
- Netondo G.W., Onyango J.C., Beck E.: Sorghum and salinity: I. Response of growth, water relations and ion accumulation to NaCl salinity. – *Crop Sci.* **44**: 797-805, 2004a.
- Netondo G.W., Onyango J.C., Beck E.: Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. – *Crop Sci.* **44**: 806-811, 2004b.
- Oukarroum A., El Madidi S., Schansker G., Strasser R.J.: Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll *a* fluorescence OLKJIP under drought stress and re-watering. – *Environ. Exp. Bot.* **60**: 438-446, 2007.
- Pennisi E.: How sorghum withstands heat and drought. – *Science* **323**: 573, 2009.
- Prasad P.V.V., Pisipati S.R., Mutava R., Tuinstra M.R.: Sensitivity of grain sorghum to high temperature stress during reproductive development. – *Crop Sci.* **48**: 1911-1917, 2008.
- Rastogi A., Stróżecki M., Kalaji H.M. *et al.*: Impact of warming and reduced precipitation on photosynthetic and remote sensing properties of peatland vegetation. – *Environ. Exp. Bot.* **160**: 71-80, 2019b.
- Rastogi A., Zivcak M., Tripathi D.K. *et al.*: Phytotoxic effect of silver nanoparticles in *Triticum aestivum*: Improper regulation of photosystem I activity as the reason for oxidative damage

- in the chloroplast. – *Photosynthetica* **57**: 209-216, 2019a.
- Srivastava B., Guisse H., Greppin, Strasser R.J.: Regulation of antenna structure and electron transport in photosystem II of *Pisum sativum* under elevated temperature probed by the fast polyphasic chlorophyll *a* fluorescence transient: OKJIP. – *BBA-Bioenergetics* **1320**: 95-106, 1997.
- Strasser R.J.: The grouping model of plant photosynthesis. – In: Akoyunoglou G., Argyroudi-Akoyunoglou J. (ed.): *Chloroplast Development*. Pp. 513-524. Elsevier/North-Holland Biomedical, Amsterdam 1978.
- Strasser R.J.: The grouping model of plant photosynthesis: Heterogeneity of photosynthetic units in thylakoids. – In: Akoyunoglou G. (ed.): *Photosynthesis III. Structure and Molecular Organisation of Photosynthetic Apparatus*. Pp. 727-737. Balaban International Science Service, Philadelphia 1981.
- Strasser R.J., Tsimilli-Michael M., Qiang S., Goltsev V.: Simultaneous *in vivo* recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. – *BBA-Bioenergetics* **1797**: 1313-1326, 2010.
- Sudhir P., Murthy S.D.S.: Effects of salt stress on basic processes of photosynthesis. – *Photosynthetica* **42**: 481-486, 2004.
- Wungrampha S., Joshi R., Singla-Pareek S.L., Pareek A.: Photosynthesis and salinity: are these mutually exclusive? – *Photosynthetica* **56**: 366-381, 2018.
- Yamaura Y., Narita A., Kusumoto Y. *et al.*: Genomic reconstruction of 100 000-year grassland history in a forested country: population dynamics of specialist forbs. – *Biol. Lett.-UK* **15**: 20180577, 2019.
- Zivcak M., Brestic M., Olsovska K., Slamka P.: Performance index as a sensitive indicator of water stress in *Triticum aestivum*. – *Plant Soil Environ.* **54**: 133-139, 2008.