DOI: 10.32615/ps.2020.025 PHOTOSYNTHETICA 58 (SI): 608-621, 2020

Special issue in honour of Prof. Reto J. Strasser

Photosynthetic activity as assessed *via* chlorophyll *a* fluorescence suggests a role of potassium channels in root to shoot signaling

O.V. VOITSEKHOVSKAJA[*,+], V.I. APOLLONOV[*], A.V. MURTUZOVA[*], C.K. RABADANOVA[*], M.A. CHARNYSH[**], I.V. DROZDOVA[***], A.I. BELYAEVA[***], O.N. KOVALEVA[#], I.G. LOSKUTOV[#], K. PAWLOWSKI[##], V.V. DEMIDCHIK[**], and E.V. TYUTEREVA[*]

Laboratory of Molecular and Ecological Physiology, Komarov Botanical Institute, Russian Academy of Sciences, Professora Popova 2, 197376 St.-Petersburg, Russia[*]
Department of Plant Cell Biology and Bioengineering, Biological Faculty, Belarusian State University, Independence Avenue 4, 220030, Minsk, Belarus[**]
Laboratory of Ecology of Plant Communities, Komarov Botanical Institute, Russian Academy of Sciences, Professora Popova 2, 197376 St.-Petersburg, Russia[***]
Department of Genetic Resources of Oat, Barley and Rye, N.I. Vavilov Institute of Plant Genetic Resources (VIR), 44 Bolshaya Morskaya Str., 190000 St.-Petersburg, Russia[#]
Department of Ecology, Environment and Plant Sciences, Stockholm University, 106 91 Stockholm, Sweden[##]

Abstract

Potassium is indispensable for plant growth. Recently, a role of K^+ channels has emerged in sensing and transducing stress and nutrient status. Tetraethylammonium (TEA^+) is a specific blocker of K^+ transport and affects K^+ channel gene expression. Two barley varieties with contrasting salinity tolerance, and a chlorophyll *b*-less mutant, were grown either in the presence of TEA^+ alone or combined with NaCl, at two different concentrations of external K^+ and Ca^{2+}, and were analyzed nine days after germination. Chlorophyll *a* transients monitored *via* JIP-tests were used to evaluate the state of the photosynthetic machinery. In contrast to reported responses to K^+ deficiency, TEA^+ inhibited shoot growth while inducing root growth and increasing photosynthetic performance. Both TEA^+ and NaCl induced the appearance of negative K-bands in OJIP kinetics and an increase in PI_{ABS}, indicating a stimulation of photosynthesis by increased sink strength in the context of root to shoot signaling.

Additional key words: *chlorina f2*; ion content; quaternary ammonium salts; salt stress.

Introduction

Potassium is the most abundant intracellular ion and osmoticum in plants. It is essential for ion balance, high photosynthetic efficiency, enzymatic activities, stress responses and water movement (Leigh and Wyn Jones 1984, Demidchik 2014). Maintaining a high potassium status is crucial for higher plants under stress, such as salinity, drought and others (Shabala and Cuin 2008). After its uptake by roots, K^+ is loaded into the xylem and transported with the transpiration stream to the shoot. There, it reaches photosynthesizing source leaves, enters the phloem, and can be translocated to sink organs (growing leaves), or recirculated back to roots. The phloem-mobile K^+ pool can be considered as 'decentralized store' of energy fueling membrane transport processes (Dreyer *et al.* 2017). Uptake of K^+ is the main driving force for seedling growth; moreover, the availability of this ion affects plant

Received 30 October 2019, *accepted* 16 March 2020.
[+]Corresponding author; e-mail: ovoitse@binran.ru
Abbreviations: ABA – abscisic acid; ABS – photon flux absorbed by the antenna of PSII units; DI – part of photon flux absorbed by the antenna of PSII units which is dissipated in PSII antenna in processes other than trapping; ET – rate of electron transport from the reduced Q_A to the intersystem electron acceptors; F_0 – minimum Chl *a* fluorescence; F_M – maximum Chl *a* fluorescence; I step – Chl *a* fluorescence at ~ 30 ms; J step – Chl *a* fluorescence at ~ 2 ms; K step – Chl *a* fluorescence at ~ 0.3 ms; NPQ – nonphotochemical quenching of the excited states of Chl; NSCC – nonselective cation channels; OEC – oxygen-evolving complex; P_{680} – reaction center Chls of PSII; PCD – programmed cell death; PI – performance index; PMF – proton motive force; PQ – plastoquinone; QAS – quaternary ammonium salts; ROS – reactive oxygen species; TEA^+ – tetraethylammonium; TR – flux of exciton trapping by active PSII reaction centers leading to Q_A reduction.
Acknowledgements: We are grateful to Sergey Khrushchev (Moscow State University, Russian Federation) for his help with *pyPhotoSyn* software, to Igor Pottosin (Colima University, Mexico) and Vasiliy Terentyev (Pushchino Scientific Centre, Russian Federation) for helpful discussions, and to Alison M. Berry (UC Davis, CA, USA) for critically reading the manuscript. Financial support of the Russian Science Foundation (18-16-00074) to E.V. Tyutereva is gratefully acknowledged.

responses to diseases (reviewed by Zörb *et al.* 2014). Potassium has also been revealed to play an important role in the metabolic switch between catabolic and anabolic programs which depends on its cytosolic concentrations, particularly under stress conditions (Demidchik *et al*. 2003, 2018; Shabala 2017). Stress-induced loss of K^+ from cells can trigger growth arrest, inhibition of biosynthetic pathways, and activation of catabolism, and, in the long run, lead to the onset of programmed cell death (PCD; Demidchik *et al.* 2003, 2010). Before the induction of PCD, the loss of K^+ from cells can lead to the channeling of energy to the induction of defense and detoxification programs such as autophagy (Demidchik *et al.* 2018, Rabadanova *et al.* 2018).

Potassium uptake from the soil by root cells and redistribution within tissues occurs *via* several transport systems, which include H^+/K^+ symporters, cation/H^+ antiporters, Shaker-type K^+-selective channels, and voltage-independent nonselective cation channels (NSCCs) (Demidchik 2014). Under normal soil K^+ concentrations (0.1–10 mM), passive flux *via* K^+-selective channels forms a major pathway for K^+ transmembrane transport which dominates K^+ influx and efflux within roots and leaves. Intriguingly, these channels can be inhibited by a highly specific blocker tetraethylammonium (TEA^+), providing a tool to chemically manipulate K^+ nutrition and redistribution in plants. Some K^+-permeable NSCCs are also sensitive to TEA^+ but to a much lesser extent (Demidchik *et al.* 2002).

As K^+ is the main osmolyte, the regulation of its levels is linked to the plant water status and leaf water-use efficiency *via* several K^+ channel-dependent control mechanisms. For example, in roots of rice, both the expression of the gene encoding the inward-rectifying Shaker-type K^+ channel OsAKT1 and the hydraulic conductivity of the root plasma membrane decreased in response to the K^+ channel blockers TEA^+ and Cs^+ (Liu *et al.* 2006). K^+ shortage leads to retardation of phloem transport, accumulation of sucrose in leaves, a decrease of the root/shoot ratio (which contrasts with plant responses to deficiency of other nutrients; Hermans *et al.* 2006), and causes water stress (Sustr *et al.* 2019). In *Arabidopsis* seedlings, K^+ deficiency inhibits root elongation (Li *et al.* 2017). The AKT1 channel appears as the most promising candidate for a sensor of K^+ concentrations in roots (Li *et al.* 2017); earlier, a similar role had been suggested for the outward-rectifying GORK channels in root hairs (Ivashikina *et al.* 2001).

Earlier studies analyzed the effects of K^+ shortage on photosynthetic performance in the presence of other stresses such as drought (Pier and Berkowitz 1987) or salinity (Ball *et al.* 1987, Chow *et al.* 1990). Notably, the authors found that the effects on photosynthesis caused by K^+ shortage greatly exceeded those caused by simultaneously applied drought or salt stress. The major responses were a decrease of chlorophyll (Chl) contents in general and of PSII reaction centers in particular, caused mainly by the inhibition of protein biosynthesis in chloroplasts by low K^+ concentrations. Recently, Kalaji *et al.* (2014) analyzed the effects of deficiency for a range of mineral nutrients, including K and N using Chl *a* fluorescence tests on maize and tomato plants, and observed a general decrease in photosynthetic performance. The analysis of fluorescent transients is a highly informative method for estimating the influence of stress factors on photosynthesis. JIP-tests described by Reto J. Strasser (Strasser *et al.* 2000, 2004) are based on the theory of energy flow in thylakoid membranes and nowadays represent the most widely used nondestructive method for estimating PSII efficiency, photosynthetic capacity, and plant vitality. Thus, the high sensitivity of fast and noninvasive techniques based on Chl *a* fluorescence induction measurements offers a new possibility to detect and even quantify early responses of plants to abiotic stress or signaling event at the level of PSII electron transport using JIP-tests (Kalaji *et al.* 2018).

The aim of this study was to elucidate the role of root TEA^+-sensitive K^+ channels in the sink strength-dependent regulation of photosynthetic activity in barley leaves. For this purpose, we tested effects of 20 mM TEA^+Cl^-, alone or added on top of 150 mM NaCl, on seedlings of two barley varieties differing in salinity tolerance, and of a chlorophyll *b*-less barley mutant of the less NaCl-tolerant variety. JIP-tests were performed for the estimation of PSII efficiency at the level of PSII electron transport.

Materials and methods

Plant growth and morphometric analyses: Seeds of two barley cultivars, Donetskiy and Donaria Ackermanns, were obtained from the N.I. Vavilov Institute of Plant Genetic Resources (Saint Petersburg, Russia) and from the Leibniz Institut für Pflanzengenetik und Kulturpflanzenforschung (Gatersleben, Germany), respectively. Seeds of the Chl *b*-less mutant, *chlorina f2* 3613 (*clo-f2*), of the parental cultivar Donaria were a gift of Dr. Mats Hansson (Lund University, Sweden). Seeds were soaked in distilled water for 1 h and then placed on latticed polystyrol rafts which floated on the surface of a medium aerated at the rate of 250 L per hour using a membrane pump from *Schego Optimal* (Germany). The hydroponic cultures were maintained in a growth chamber under 80% relative humidity (RH). Seedlings were grown at 19–21°C with a PPFD of 80 μmol m^{-2} s^{-1} in a 16-h light/8-h dark cycle for 9 d after germination. The reference medium for hydroponic cultures was Hoagland's nutrient solution (Hoagland and Arnon 1950) with addition of $CoCl_2$:

Macroelements	[mM]
KH_2PO_4	1
KNO_3	5
$Ca(NO_3)_2 \times 4H_2O$	5
$MgSO_4 \times 7H_2O$	2
Microelements	[mg l^{-1}]
H_3BO_3	2.86
$MnCl_2 \times 4H_2O$	1.81
$ZnSO_4 \times 7H_2O$	0.22
$CuSO_4 \times 5H_2O$	0.08
$Na_2MoO_4 \times 2H_2O$	0.025
$CoCl_2 \times 6H_2O$	0.025
$FeSO_4 \times EDTA$	0.10

For all media, pH was set to 5.8. Eight different media were used:

Media
reference medium
reference medium with 20 mM TEA^+Cl^-
reference medium with 150 mM NaCl
reference medium with 150 mM NaCl and 20 mM TEA^+Cl^-
2× reference medium
2× reference medium with 20 mM TEA^+Cl^-
2× reference medium with 150 mM NaCl
2× reference medium with 150 mM NaCl and 20 mM TEA^+Cl^-

In 2× reference medium, the concentrations of all components of Hoagland's medium are doubled. Two independent experiments were performed for each growth condition; 100–200 seeds were germinated per genotype and experimental variant. Seedlings were used for analysis 9 d after germination (*i.e.*, after placing the seeds on the growth medium). Measurements of root, leaf, and coleoptile length were performed for all seedlings available per genotype and variant. Significance of the differences was evaluated using the nonparametric *Mann-Whitney U*-test at the level of $p<0.05$. Root/shoot biomass ratio was determined by weighing whole shoots and whole root systems, respectively.

Estimation of salt resistance: Two barley cultivars, Donetskiy and Donaria Ackermanns, and the Chl *b*-less mutant *chlorina f2* 3613 (Donaria background) were compared regarding their ability to tolerate salinity using the method developed by Udovenko and Volkova (1993). Briefly, 100 seeds of each genotype were germinated in the dark either on distilled water or on 168 mM NaCl (0.98%, w/v) in distilled water, or on 215 mM NaCl (1.26%, w/v) in distilled water. Root length was determined after 7 d, and the inhibition of root growth by NaCl was expressed in percent of root length of seedlings germinated on water. Based on the results of these tests, barley cultivars were separated into salt tolerant (less than 40% inhibition), moderately salt tolerant (40−60% inhibition) or salt sensitive (more than 60% inhibition of root growth). Statistical significance of the differences was estimated using 30 seedlings per genotype.

Chl *a* fluorescent transients: Fluorescence measurements were performed using a *DUAL-PAM 100* (*Walz*, Germany) on 8–24 seedlings per genotype. Seedlings were dark-adapted overnight prior to analyses. OJIP measurements were performed using saturating red light of 3,000 μmol(photon) m^{-2} s^{-1}, pulse width 80 μs and 300 ms polyphasic fluorescence trigger mode. Fluorescence transients were recorded with a data acquisition rate of 1 per 10 μs. The fluorescence signal at 70 μs after the onset of illumination was considered as F_0. Recorded *DUAL-PAM 100* data in the CSV format were analysed using *pyPhotoSyn* software (Plyusnina *et al.* 2015). Several parameters were determined from the polyphasic Chl *a* fluorescence OJIP rise (Stirbet *et al.* 2018). Performance index on absorption basis (PI_{ABS}) was determined as $(RC/ABS) \times [\psi_{E0}/1 - \psi_{E0})] \times [\varphi_{P0}/(1 - \varphi_{P0})]$, where RC/ABS is the density of PSII reaction centers per absorption basis calculated as $\varphi_{P0} \times V_J/M_0$. $\psi_{E0} = 1 - V_J$, where V_J is the relative variable fluorescence in J phase $V_J = (F_J - F_0)/F_J$. ψ_{E0} is the efficiency with which a PSII trapped electron is transported beyond Q_A^- (Stirbet *et al.* 2018). φ_{P0} ($= F_V/F_M$) is the maximum quantum yield of primary PSII photochemistry.

Further parameters determined for specific energy fluxes (per Q_A-reducing PSII reaction center RC) were: $ABS/RC = (M_0/V_J)/\varphi_{P0}$ – absorption flux (of antenna Chls) per RC; $TR_0/RC = M_0/V_J$ – trapping flux (leading to Q_A reduction) per RC; $ET_0/RC = (M_0/V_J) \times \psi_{E0}$ – electron transport flux (further than Q_A^-) per RC; $DI_0/RC = ABS/RC - TR_0/RC$ – the flux of energy dissipated in processes other than trapping per active PSII, where $M_0 = (\Delta V/\Delta t)_0 \approx 4(F_{300\mu s} - F_{50\mu s})/F_V$ – approximated initial slope (in ms^{-1}) of the fluorescence transient normalized on the maximal variable fluorescence F_V. This parameter also expresses the rate of accumulation of closed reaction centers.

Analysis of nonphotochemical quenching (NPQ): Nine-day-old barley seedlings (10 seedlings per genotype and experimental variant) were dark-adapted for 1 h. Fluorescence measurements were performed using a *DUAL-PAM 100*. The measuring light was turned on, and after 5 s, a saturating light pulse of 3,000 μmol(photon) m^{-2} s^{-1} was given for 800 ms to estimate F_0 and F_M. After 50-s exposure to darkness, actinic red light [800 μmol(photon) m^{-2} s^{-1}] was given for 12 min. During this period, saturating light pulses of 3,000 μmol(photon) m^{-2} s^{-1} for 800 ms were administered every 50 s. Afterwards, the actinic light was changed to 90 μmol(photon) m^{-2} s^{-1} and the relaxation kinetic was estimated over the next 12 min by applying saturating light pulses with the same parameters as described above. NPQ was calculated according to the formula $NPQ = (F_M - F_M')/F_M'$.

To estimate the direct effect of TEA^+ on NPQ, leaves of barley seedlings were cut off, placed bottom-side-down in glass beakers containing either 20 mM TEA^+Cl^- in distilled water or dH_2O and incubated in the dark overnight (for *ca.* 10 h). After this, NPQ analysis was performed as described above.

Chl contents: Leaves of barley seedlings were extracted with 96% ethanol at 65°C. Chl contents in the extracts were determined using a *UV-2401 PC* spectrophotometer (*Shimadzu*, Japan) according to Lichtenthaler and Wellburn (1983). Ten seedlings were analyzed per genotype and variant.

K^+ and Na^+ concentrations: Leaf samples were dried at 100°C and transferred to acid-washed quartz beakers which were placed in a muffle furnace at 450°C and then transferred into a desiccator for cooling. The ash was dissolved in a mixture of 1.5 M HNO_3 and 3.71 M HCl. The concentrations of K^+ and Na^+ were determined

using atomic absorption spectrophotometer *Quantum-AFA* (*Cortec*, Russia). Performance of the instrument was checked by analyzing the reference standard material solutions: State Standard Samples GSO 8092–94 and GSO 8062–94. For each genotype, five leaves from five different seedlings were analyzed.

Determination of osmolality in root and leaf sap: Seedling roots or leaves (20−100 mg each) were harvested, placed in 0.5-ml Eppendorf tubes with a hole in the bottom and frozen at –20°C, then placed in 1.5-ml tubes, thawn and centrifuged at 10,000 rpm at 4°C for 5 min to extract the cell sap. This sap was used for the determination of the osmolality using *Vapro 5600* osmometer (*Wescor*, USA). Ten seedlings were analyzed per genotype and variant.

Statistical analysis: For plant growth and morphometric analyses, significance of the differences was evaluated using the nonparametric *Mann-Whitney U*-test at the level of $p<0.05$. For estimation of salt resistance, determination of chlorophyll contents, determination of K^+ and Na^+ concentrations and osmolality, statistical significance of the differences was estimated using *Student*'s *t*-test at the level of $p<0.05$. For NPQ analysis, statistical significance of the differences was estimated using one-way analysis of variance (*ANOVA*) at the level of $p<0.05$.

Results

Tetraethylammonium chloride-dependent inhibition of shoot growth and modification of NaCl-induced effects: Salt stress tolerance tests of barley genotypes showed that the cultivar Donetskiy was salt tolerant (24–30% inhibition of root growth was induced by both NaCl concentrations that were applied), while both Donaria and its mutant *clo-f2* were only moderately salt tolerant (41–47% inhibition of root growth for Donaria and 41–50% for *clo-f2*, respectively). TEA^+ (20 mM) strongly affected growth of seedlings in reference medium. Root elongation was significantly increased by TEA^+ in Donaria and *clo-f2* (Fig. 1), while shoot growth was strongly inhibited in all three genotypes studied (Fig. 1 for leaves; data not shown for coleoptiles). This effect on growth was also reflected by the root/shoot ratios determined on the basis of fresh mass (Fig. 1), which increased strongly in seedlings grown in the presence of TEA^+ compared to control. When germinated and grown in the presence of 150 mM NaCl, all genotypes exhibited a dramatic reduction of germination rate (decrease by 80–90%; data not shown) and growth (Fig. 1). The effect of NaCl on shoot growth was much stronger than the effect on root growth, leading to an increase in the root/shoot ratio, similar to the effect of TEA^+ (Fig. 1). Addition of 20 mM TEA^+ on top of 150 mM NaCl led to an even more dramatic reduction of the germination rate (a decrease by 90% in Donetskiy and *clo-f2*, and an even stronger decrease in Donaria; data not shown) and growth (Fig. 1). Analysis of the effect of the combination of TEA^+ and NaCl on the root/shoot ratio was not possible as most seedlings did not show any shoot growth.

Notably, the percentages of germinated and growing seedlings on TEA^+, NaCl, and NaCl + TEA^+ differed significantly between three genotypes studied. Only 74% of Donetskiy seedlings continued growth on reference medium supplemented with TEA^+, while 100% seedlings of Donaria and *clo-f2* continued growth under the same conditions. However, only 5% of Donaria seedlings continued growth on 150 mM NaCl, while 51% of the Donetskiy and 65% of the *clo-f2* seedlings did. In the presence of 20 mM TEA^+ combined with 150 mM NaCl, only 35% of the seedlings of Donetskiy, but 73 % of the *clo-f2* seedlings continued growth while practically all germinated seeds of Donaria stopped further growth.

Lack of tetraethylammonium chloride effects on shoot K^+ and Na^+ concentrations and on leaf sap osmolality: In barley seedlings grown on reference medium supplemented with 20 mM TEA^+, leaf sap osmolality was not changed as compared to control seedlings, but an increase in the osmolality of the root sap was observed for all genotypes studied (Fig. 2*A*). However, growth in the presence of 150 mM NaCl, or of NaCl plus TEA^+, led to a strong increase in the osmolalities of both leaf and root sap as compared to growth on reference medium (Fig. 2*A*). Interestingly, germination and growth in the presence of 20 mM TEA^+ did not affect the concentrations of K^+ in the shoots, except for Donetskiy seedlings where a drop of shoot K^+ concentrations by 20% was observed as compared to shoot K^+ contents in the seedlings grown on the reference medium. However, K^+ concentrations in the roots were reduced by 50–55% in Donetskiy and Donaria, and by *ca.* 30% in *clo-f2* (Fig. 2*C*). Growth in the presence of 150 mM NaCl led to accumulation of Na^+ in shoots and roots (Fig. 2*B*,*C*); notably, Na^+ concentrations in shoots of salt-tolerant Donetskiy seedlings were significantly lower than those of the moderately salt-tolerant *clo-f2* mutant (Fig. 2*B*). Due to a decrease in germination rate and growth in the presence of 150 mM NaCl and 20 mM TEA^+, the contents of shoot and root K^+ and Na^+ could be only determined for *clo-f2* under these conditions (Fig. 2*B*,*C*); here, TEA^+ had no significant influence on Na^+ accumulation. In Donetskiy and *clo-f2* grown on medium containing NaCl, as well as in *clo-f2* grown in the presence of both NaCl and TEA^+, the shoot ratios of K^+/Na^+ were similar (*ca*. 1), while in roots, K^+ contents exceeded Na^+ contents; a determination of the K^+/Na^+ ratios for Donaria was not possible due to the stronger inhibition of germination and growth of this genotype by both NaCl and NaCl plus TEA^+.

Tetraethylammonium chloride- or NaCl-dependent effects on electron transport at PSII: For analysis of fast Chl fluorescence kinetics, we carried out two series of experiments. In the first series, seedlings were grown on 1× Hoagland's reference medium (control) or on medium supplemented with 20 mM TEA^+ and/or 150 mM NaCl as described above. In the second series of experiments, seedlings were grown on 2× Hoagland's reference medium (control) or the same medium supplemented with 20 mM TEA^+ and/or 150 mM NaCl. Thus, in the first series of experiments, the external concentrations of K^+ and Ca^{2+} were 6 and 5 mM, respectively, while in the second series,

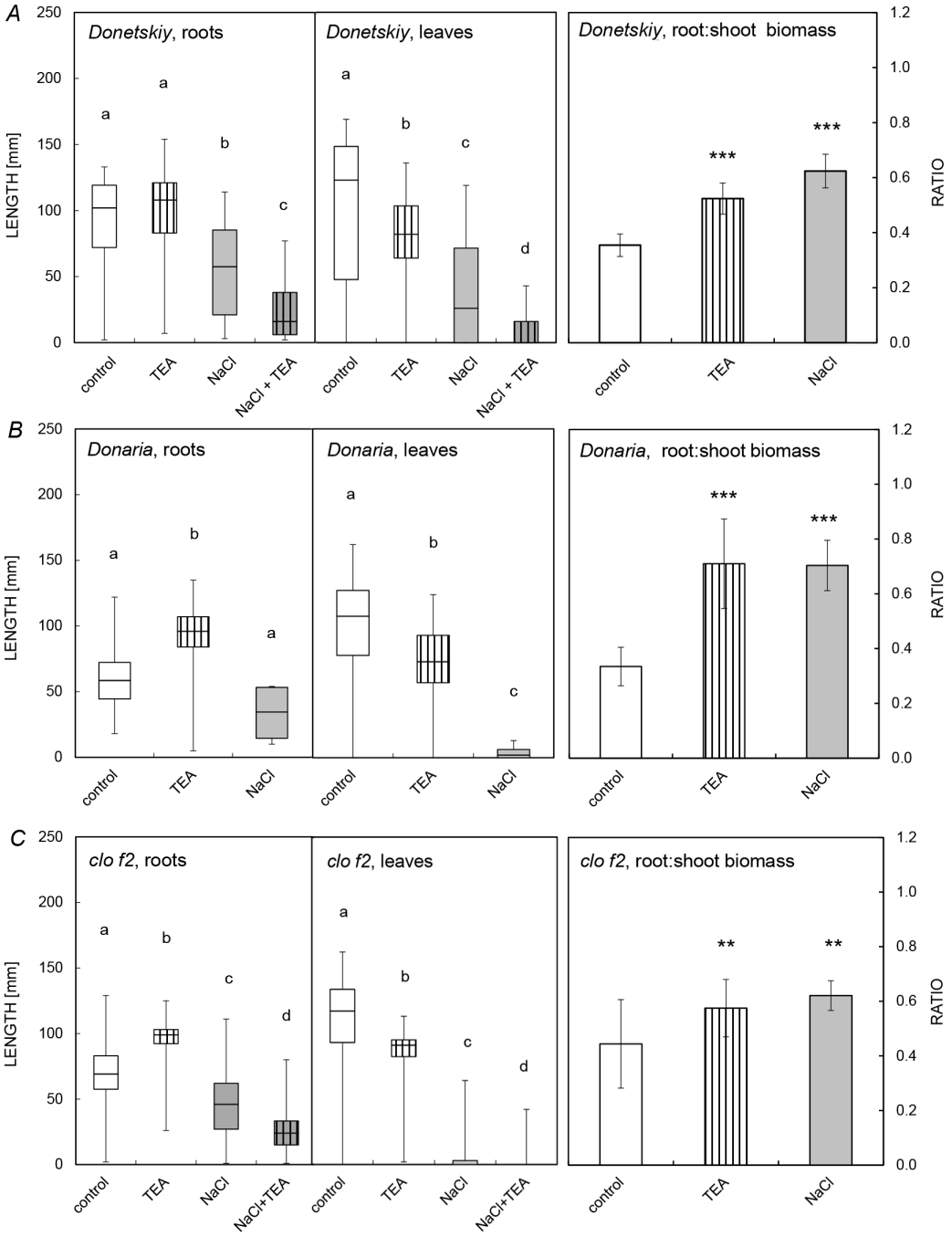

Fig. 1. Effects of TEA^+Cl^- (20 mM), NaCl (150 mM) or their combination on growth of roots and leaves, and on root/shoot ratio, in barley seedlings of Donetskiy (*A*), Donaria (*B*), and *clo-f2* (*C*). Control: values obtained for seedlings grown on 1× Hoagland's medium. For length of roots and leaves, the diagrams show the minimum value, the first quartile, the median, the third quartile, and the maximum value of 100 independent measurements (seedlings) per variant and genotype. *Different letters* indicate significant differences at $p<0.05$ according to *Mann-Whitney U*-test. For root/shoot ratio, the data show mean values ± SD; ** and *** indicate significant differences relative to control at $p<0.01$ and $p<0.001$, respectively, according to *Student*'s *t*-test.

they were 12 and 10 mM, respectively. When 2× Hoagland's reference medium was used, TEA^+ induced a statistically significant decrease in shoot length but no effect on root length, while the increase in root/shoot ratio was much less pronounced than that in the first series of experiments that had been based on 1× Hoagland's medium (data not shown).

For both series, typical OJIP transients were observed when fast Chl fluorescence rise was measured and plotted on a logarithmic time scale (Fig. 3*A*,*B*). Fluorescence data were normalized between the steps O and J (2 ms) as $W_{OJ} = (F_t - F_0)/(F_J - F_0)$, and plotted as difference kinetics $\Delta W_{OJ} = W_{OJ(experiment)} - W_{OJ(control)}$ between 'experimental'

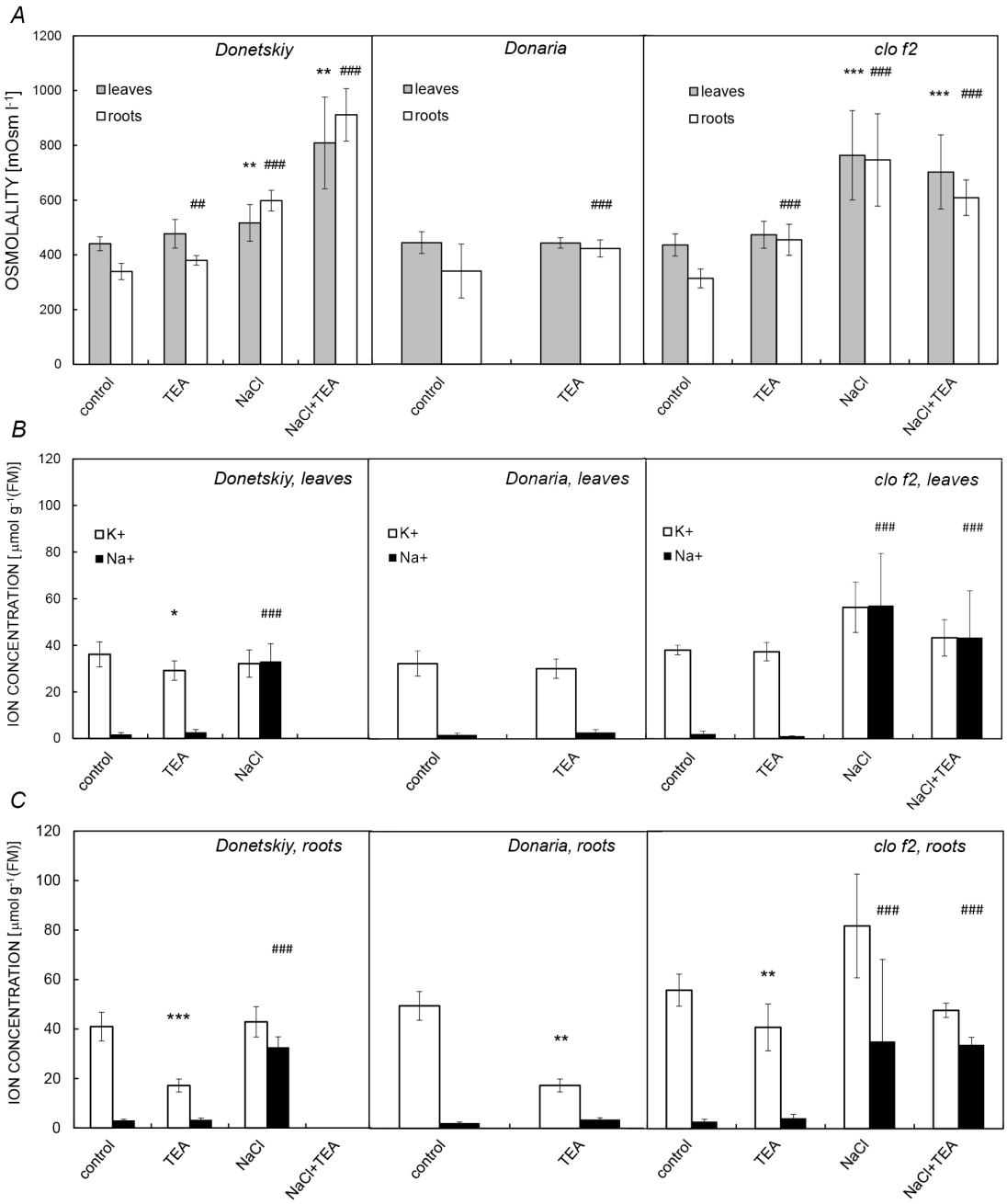

Fig. 2. Effects of TEA^+Cl^- (20 mM), NaCl (150 mM) or their combination on osmolality of leaf and root sap (*A*), and the levels of K^+ and Na^+ in shoots (*B*) and roots (*C*) of barley seedlings. Control: values obtained for seedlings grown on 1× Hoagland's medium. Mean values ± SD for 10 (*A*) or 5 (*B*,*C*) independent measurements (seedlings) are shown. *, ** and *** indicate significant differences at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, for leaf sap (*A*) and for K^+ shoot (*B*) and root (*C*) levels. $^{\#\#}$ and $^{\#\#\#}$ indicate significant differences at $p<0.01$ and $p<0.001$, respectively, for root sap (*A*) and for Na^+ shoot (*B*) and root (*C*) levels.

values obtained for seedlings grown on experimental media minus 'control' values for seedlings grown on 1× Hoagland's reference medium (Fig. 3*C*). For this calculation, 2× Hoagland's reference medium was also considered as an experimental medium. In all cases except for seedlings grown on 2× Hoagland's medium, negative K-bands were observed. The amplitude of the K-bands increased in the following order: 2× Hoagland's + TEA^+ < 1× Hoagland's + TEA^+ < 2× Hoagland's + NaCl < 1× Hoagland's + NaCl ≃ 1× Hoagland's + NaCl +TEA^+ ≃ 2× Hoagland's + NaCl + TEA^+ (Fig. 3*C*).

The OJIP transients were further translated to biophysical parameters using JIP-test equations; some of these parameters, namely, quantum yield φ_{P0}, specific activities per reaction center, and performance index on absorption basis (potential for energy conservation from

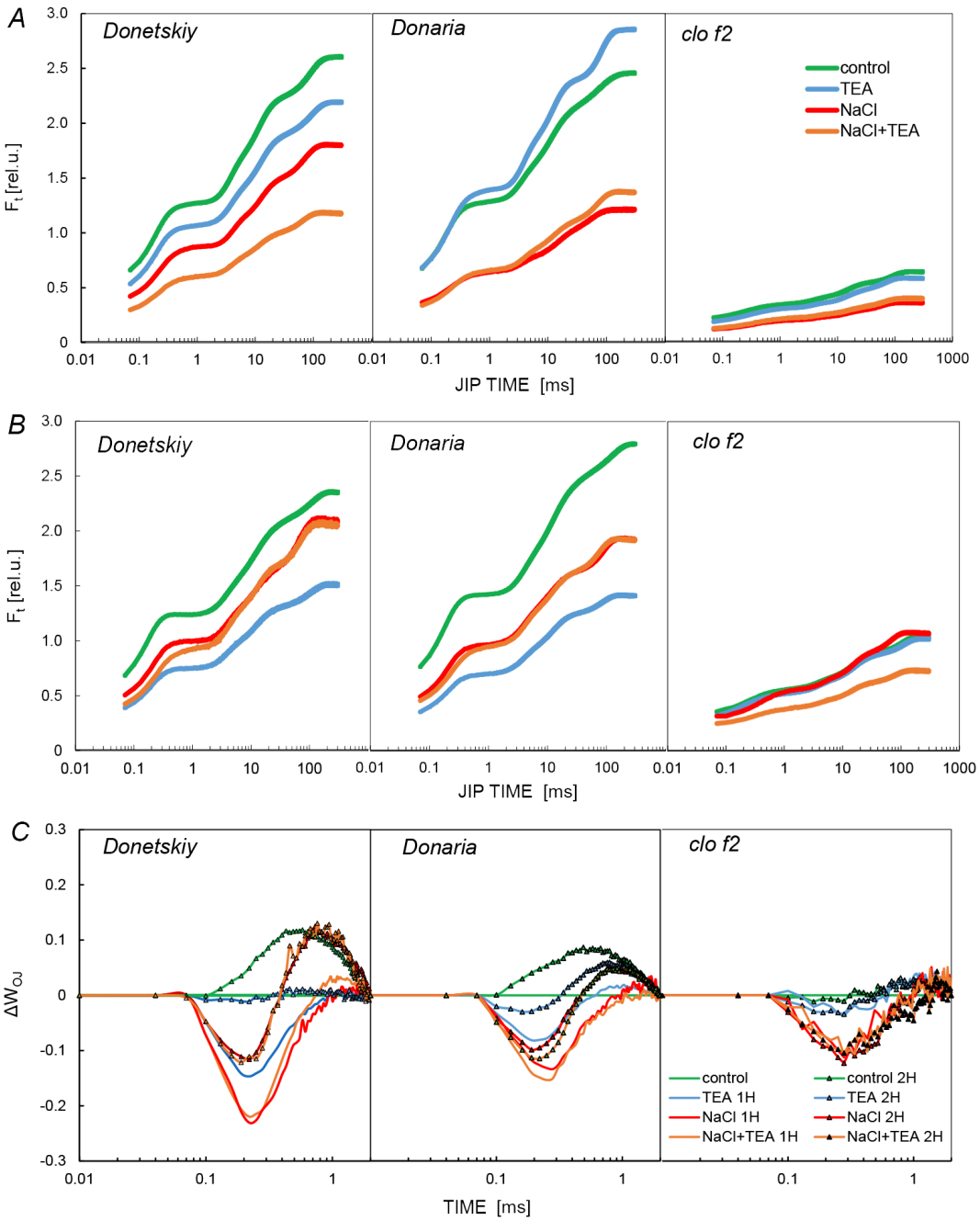

Fig. 3. Effects of TEA^+Cl^- (20 mM), NaCl (150 mM) or their combination on the fluorescence induction parameters of barley seedlings. (*A*,*B*) OJIP curves obtained with seedlings grown on 1× Hoagland's medium (*A*) and 2× Hoagland's medium (*B*). 'Control' refers to seedlings grown on the reference medium. Each curve is the average of 8–24 replicates. (*C*) The shape of K-bands. Chlorophyll *a* fluorescent transients were normalized between F_0 and F_J and expressed as $\Delta W_{OJ} = W_{OJ(experiment)} - W_{OJ(control)}$, where $W = (F_t - F_0)/(F_J - F_0)$, 'control' refers to seedlings grown on 1× Hoagland's medium and 'experiment' to other treatments. Control 2H – seedlings grown on 2× Hoagland's medium; TEA 1H – seedlings grown on 1× Hoagland's medium supplemented with TEA^+; NaCl 1H – seedlings grown on 1× Hoagland's medium supplemented with NaCl; NaCl+TEA 1H – seedlings grown on 1× Hoagland's medium supplemented with NaCl and TEA^+; TEA 2H – seedlings grown on 2× Hoagland's medium supplemented with TEA^+; NaCl 2H – seedlings grown on 2× Hoagland's medium supplemented with NaCl; NaCl+TEA 2H – seedlings grown on 2× Hoagland's medium supplemented with NaCl and TEA^+. Each curve is the average of 8–24 replicates.

exciton to the reduction of intersystem electron acceptors) are listed in Tables 1 and 2.

Chl concentrations and Chl *a*/*b* ratio were determined for seedlings grown on 1× Hoagland's medium (Table 1). In the salt-tolerant Donetskiy genotype, addition of TEA^+ or NaCl induced strong accumulation of Chl *a*, while

Table 1. Chlorophyll (Chl) contents and photosynthetic performance (JIP-tests parameters) of dark-adapted barley seedlings. Seedlings grown on 1× Hoagland's medium were used as reference. Data are from two independent experiments. *, ** and *** indicate statistically significant differences to the reference conditions (1× Hoagland's) according to *Student*'s *t*-test at the levels of $p<0.05$, $p<0.01$ and $p<0.001$, respectively. F_0 – minimum Chl *a* fluorescence; F_M – maximum Chl *a* fluorescence; φ_{P0} – the maximum quantum yield of primary PSII photochemistry; PI_{ABS} – performance index on absorption basis; ABS/RC – absorption flux of antenna Chls per RC; TR_0/RC – trapping flux (leading to Q_A reduction) per RC; DI_0/RC – the flux of energy dissipated in processes other than trapping per active PSII; ET_0/RC – electron transport flux (further than Q_A^-) per RC; M_0 – approximated initial slope (in ms^{-1}) of the fluorescence transient normalized on the maximal variable fluorescence.

	1× Hoagland's	TEA^+	NaCl	NaCl + TEA^+
Donetskiy				
Chl *a* [mg g^{-1}(FM)]	0.73 ± 0.16	0.92 ± 0.12***	1.17 ± 0.21***	0.75 ± 0.18
Chl *b* [mg g^{-1}(FM)]	0.23 ± 0.05	0.26 ± 0.05	0.34 ± 0.06*	0.28 ± 0.05
Chl *a*/*b*	3.3 ± 0.6	3.5 ± 0.4	3.4 ± 0.2	2.7 ± 0.2*
F_0	0.46 ± 0.10	0.40 ± 0.08**	0.39 ± 0.10*	0.35 ± 0.13***
F_M	2.48 ± 0.56	2.27 ± 0.46	2.27 ± 0.62	2.00 ± 0.83***
φ_{P0}	0.81 ± 0.02	0.83 ± 0.01**	0.79 ± 0.15	0.75 ± 0.21
PI_{ABS}	1.688 ± 0.368	1.927 ± 0.216***	1.922 ± 0.505	1.874 ± 0.620
ABS/RC	3.937 ± 0.309	3.747 ± 0.307**	3.561 ± 0.741***	3.212 ± 0.874***
TR_0/RC	3.197 ± 0.264	3.096 ± 0.255*	2.935 ± 0.619***	2.628 ± 0.721***
DI_0/RC	0.741 ± 0.105	0.651 ± 0.057***	0.626 ± 0.151	0.585 ± 0.183
ET_0/RC	1.908 ± 0.158	1.858 ± 0.144	1.766 ± 0.373**	1.578 ± 0.440***
M_0	1.289 ± 0.156	1.238 ± 0.122	1.169 ± 0.251***	1.051 ± 0.288**
Donaria				
Chl *a* [mg g^{-1}(FM)]	0.74 ± 0.12	0.72 ± 0.06	n.d.	n.d.
Chl *b* [mg g^{-1}(FM)]	0.21 ± 0.09	0.17 ± 0.08	n.d.	n.d.
Chl *a*/*b*	3.5 ± 0.5	4.0 ± 1.4	n.d.	n.d.
F_0	0.39 ± 0.13	0.40 ± 0.12	0.30 ± 0.11	0.25 ± 0.08**
F_M	2.18 ± 0.66	2.32 ± 0.64	1.23 ± 0.42*	1.36 ± 0.47**
φ_{P0}	0.82 ± 0.02	0.83 ± 0.01*	0.75 ± 0.02*	0.82 ± 0.02
PI_{ABS}	1.688 ± 0.322	2.050 ± 0.313***	1.345 ± 0.145**	2.341 ± 0.295***
ABS/RC	4.106 ± 0.269	3.738 ± 0.315***	3.499 ± 0.246*	3.203 ± 0.196***
TR_0/RC	3.370 ± 0.225	3.098 ± 0.258***	2.645 ± 0.252**	2.612 ± 0.170***
DI_0/RC	0.736 ± 0.093	0.641 ± 0.071***	0.855 ± 0.053*	0.592± 0.070***
ET_0/RC	2.007 ± 0.137	1.885 ± 0.142***	1.591 ± 0.145**	1.634 ± 0.078***
M_0	1.363 ± 0.120	1.213 ± 0.128***	1.053 ± 0.123*	0.977 ± 0.098***
clo-f2				
Chl *a* [mg g^{-1}(FM)]	0.38 ± 0.06	0.35 ± 0.05	0.37 ± 0.04	0.51 ± 0.06*
F_0	0.23 ± 0.08	0.20 ± 0.07**	0.14 ± 0.09**	0.11 ± 0.03***
F_M	0.77 ± 0.24	0.72 ± 0.32	0.49 ± 0.26**	0.42 ± 0.10***
φ_{P0}	0.69 ± 0.02	0.72 ± 0.02***	0.71 ± 0.03	0.73 ± 0.01**
PI_{ABS}	1.318 ± 0.275	1.674 ± 0.206***	2.043 ± 0.614**	1.918 ± 0.271**
ABS/RC	3.218 ± 0.395	2.907 ± 0.420***	2.354 ± 0.437***	2.450 ± 0.188***
TR_0/RC	2.221 ± 0.237	2.094 ± 0.388*	1.669 ± 0.325***	1.784 ± 0.146***
DI_0/RC	0.997 ± 0.171	0.812 ± 0.067***	0.685± 0.145***	0.667 ± 0.055***
ET_0/RC	1.433 ± 0.126	1.361 ± 0.206*	1.081 ± 0.185***	1.131 ± 0.080***
M_0	0.788 ± 0.117	0.734 ± 0.187	0.589 ± 0.147***	0.653 ± 0.073**

NaCl also induced an increase in Chl *b* contents. This was not found in Donaria and *clo-f2*, where Chl contents remained constant under experimental conditions. JIP-test parameters showed a general increase in photosynthetic performance per specific active RC, although the most pronounced changes were detected in seedlings grown in the presence of TEA^+, where a significant increase in φ_{P0} was observed for all varieties studied (Table 1). Specific energy fluxes per active PSII RC showed concerted changes in all barley lines under investigation, while the performance index on absorption basis, PI_{ABS}, demonstrated a significant increase in TEA^+-exposed seedlings. The rate

Table 2. Photosynthetic performance (JIP-tests parameters) of dark-adapted barley seedlings. 2× Hoagland's medium was used as a reference medium which under experimental conditions was further supplemented with TEA^+ (20 mM), NaCl (150 mM) or NaCl (150 mM) + TEA^+ (20 mM). Data are from two independent experiments. *, ** and *** indicate statistically significant differences to the values obtained on 2× Hoagland's medium according to *Student*'s *t*-test at the levels of $p<0.05$, $p<0.01$ and $p<0.001$, respectively. F_0 – minimum Chl *a* fluorescence; F_M – maximum Chl *a* fluorescence; φ_{P0} – the maximum quantum yield of primary PSII photochemistry; PI_{ABS} – performance index on absorption basis; ABS/RC – absorption flux of antenna Chls per RC; TR_0/RC – trapping flux (leading to Q_A reduction) per RC; DI_0/RC – the flux of energy dissipated in processes other than trapping per active PSII; ET_0/RC – electron transport flux (further than Q_A^-) per RC; M_0 – approximated initial slope (in ms^{-1}) of the fluorescence transient normalized on the maximal variable fluorescence.

	2× Hoagland's	TEA^+	NaCl	NaCl + TEA^+
Donetskiy				
F_0	0.49 ± 0.16	0.51 ± 0.19	0.17 ± 0.06***	0.16 ± 0.06***
F_M	2.22 ± 0.67	2.34 ± 0.89	0.79 ± 0.37***	0.77 ± 0.49***
φ_{P0}	0.78 ± 0.02	0.78 ± 0.02	0.77 ± 0.03	0.77 ± 0.03
PI_{ABS}	1.012 ± 0.190	1.217 ± 0.3206**	1.291 ± 0.389**	1.380 ± 0.681
ABS/RC	5.053 ± 0.435	4.375± 0.438***	3.222 ± 0.465***	4.040 ± 0.497***
TR_0/RC	3.912 ± 0.270	3.397 ± 0.283***	3.223 ± 0.289***	3.094 ± 0.300***
DI_0/RC	1.141 ± 0.187	0.978 ± 0.180**	0.999 ± 0.221**	0.946 ± 0.212**
ET_0/RC	2.315 ± 0.191	2.011 ± 0.139***	1.972 ± 0.157***	1.870 ± 0.191***
M_0	1.597 ± 0.115	1.385 ± 0.162***	1.251 ± 0.152***	1.223 ± 0.145***
Donaria				
F_0	0.41 ± 0.14	0.34 ± 0.07*	0.38 ± 0.19	0.48 ± 0.19
F_M	1.96 ± 0.82	1.63 ± 0.38*	2.08 ± 1.11	2.75 ± 1.15
φ_{P0}	0.78 ± 0.04	0.79 ± 0.02	0.81 ± 0.01***	0.82 ± 0.01***
PI_{ABS}	1.180 ± 0.325	1.419 ± 0.284**	1.916 ± 0.289***	2.315 ± 0.259***
ABS/RC	4.474 ± 0.355	3.938 ± 0.250***	3.446 ± 0.271***	3.246 ± 0.218***
TR_0/RC	3.475 ± 0.306	3.112 ± 0.167***	2.797 ± 0.234***	2.669 ± 0.169***
DI_0/RC	0.999 ± 0.197	0.826 ± 0.115***	0.649 ± 0.059***	0.577± 0.060***
ET_0/RC	2.044 ± 0.215	1.837 ± 0.110***	1.681 ± 0.115***	1.647 ± 0.158***
M_0	1.431 ± 0.146	1.275 ± 0.079***	1.116 ± 0.130***	1.022 ± 0.063***
clo-f2				
F_0	0.50 ± 0.15	0.54 ± 0.14	0.28 ± 0.11***	0.29 ± 0.10***
F_M	1.66 ± 0.59	1.89 ± 0.48	0.93 ± 0.40***	1.00 ± 0.34***
φ_{P0}	0.68 ± 0.03	0.71 ± 0.01***	0.70 ± 0.02	0.71 ± 0.02**
PI_{ABS}	1.442 ± 0.251	1.780 ± 0.389***	1.623 ± 0.488	2.011 ± 0.482*
ABS/RC	3.144 ± 0.301	2.872 ± 0.252***	2.788 ± 0.422**	2.402 ± 0.455***
TR_0/RC	2.1481 ± 0.155	2.042 ± 0.167*	1.932 ± 0.249*	1.770 ± 0.277*
DI_0/RC	0.997 ± 0.168	0.830 ± 0.094***	0.856± 0.185**	0.732 ± 0.185***
ET_0/RC	1.442 ± 0.120	1.365 ± 0.105**	1.258 ± 0.148***	1.168 ± 0.155**
M_0	0.706 ± 0.056	0.677 ± 0.071	0.673 ± 0.110	0.602 ± 0.126

of accumulation of closed reaction centers (as judged from the M_0 parameter) decreased significantly in response to NaCl, but much less in response to TEA^+ alone. Altogether, on 1× Hoagland's medium, TEA^+ caused an increase in Chl *a* contents in Donetskiy and in PSII performance in all genotypes studied, while similar patterns but somewhat weaker trends were observed for seedlings grown in the presence of NaCl +/– TEA^+.

For the second series (with 2× Hoagland's reference medium), a similar pattern but weaker trend was observed. For Donetskiy, no effect of treatments on φ_{P0} was detected while for *clo-f2*, an increase was found in the presence of TEA^+. In the presence of NaCl + TEA^+, φ_{P0} increased in both Donaria and *clo-f2*. In all three genotypes, PI_{ABS} increased in all three treatments compared to the reference medium, and in the two wild types, the increase was more pronounced in the presence of NaCl than in the presence of TEA^+ alone (Table 2).

Modification of establishment and relaxation of NPQ: Potassium is important for the transport of H^+ into and out of thylakoid lumen, affecting establishment and relaxation of NPQ. Analysis of the development of NPQ showed a significant increase in the rate of NPQ establishment in seedlings grown in the presence of TEA^+, NaCl, or their combination when the seedlings were exposed to high

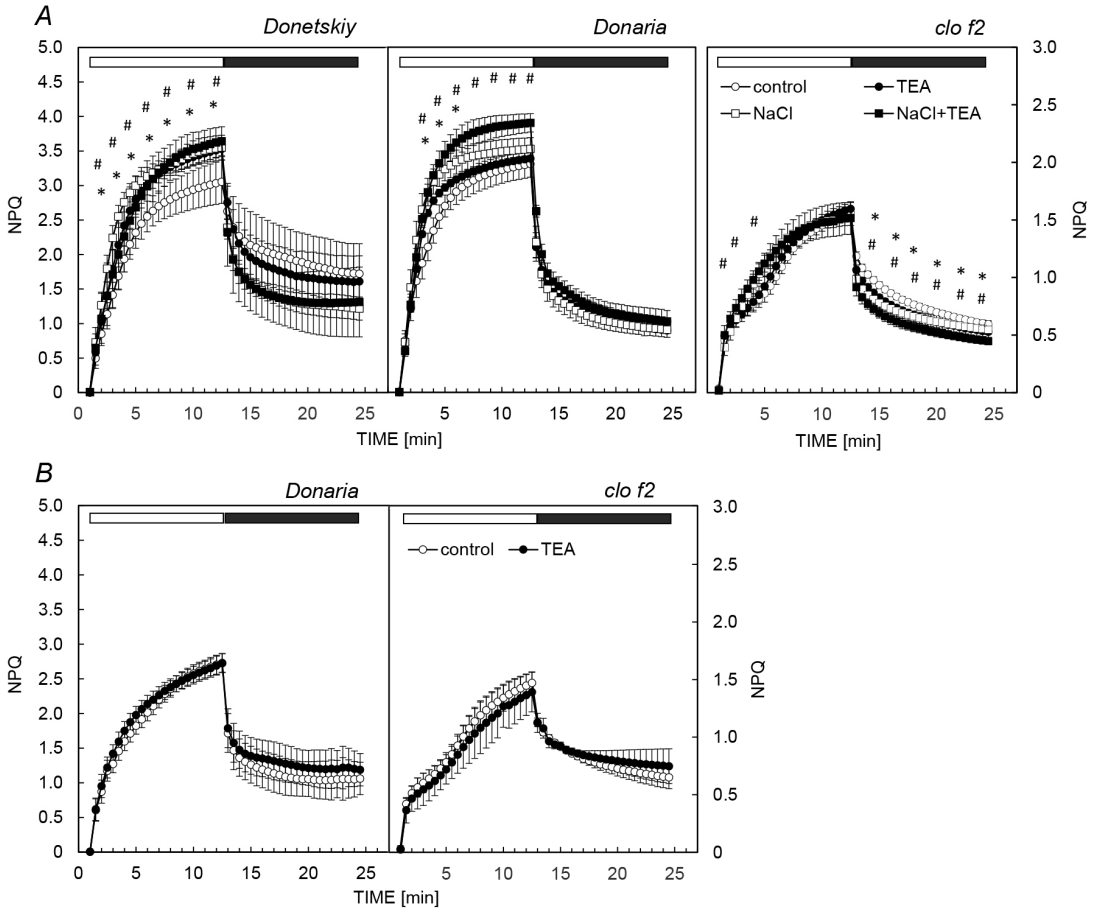

Fig. 4. (*A*) Effect of TEA^+Cl^- (20 mM), NaCl (150 mM) or their combination on the NPQ development in barley seedlings at high light (800 µmol m^{-2} s^{-1}) and on the relaxation of NPQ after the lowering of light intensity to 90 µmol m^{-2} s^{-1}. (*B*) Effect of feeding TEA^+Cl^- (20 mM) overnight with transpiration stream to detached leaves of Donaria and *clo-f2* on the development and relaxation of NPQ under conditions similar to (*A*). Each point corresponds to the average of 3–5 independent measurements. Mean values ± SD are shown. * and # indicate significant differences at $p<0.05$ between 'control' and 'TEA' and 'control' and 'NaCl', respectively, according to one-way *ANOVA*.

light [800 µmol(photon) m^{-2} s^{-1}; Fig. 4*A*]. When light levels were lowered from 800 to 90 µmol(photon) m^{-2} s^{-1}, no differences in the rate of NPQ relaxation could be observed between genotypes except for *clo-f2*. In *clo-f2,* relaxation of NPQ was significantly faster in the seedlings exposed to TEA^+ than that in the controls (Fig. 4*A*).

TEA^+ had been reported to be able to enter plants to some extent, but no data on root-to-shoot translocation *via* the xylem are available (Demidchik and Tester 2002). Thus, potential direct effects of TEA^+ on K^+ transport within chloroplasts and on the expression levels of K^+ channel encoding genes could not be excluded. To address this quandary, leaves of two genotypes grown in reference medium, Donaria and *clo-f2,* were detached, placed in 20 mM TEA^+ dissolved in distilled water and incubated in the dark overnight; control leaves were incubated in distilled water without additives. Analyses of the NPQ dynamics of both groups did not reveal any differences between water-fed and TEA^+-fed leaves of both genotypes under either 800 or 90 µmol(photon) m^{-2} s^{-1} light (Fig. 4*B*). Thus, the effects observed in whole seedlings could not be due to uptake and root-to-shoot translocation of TEA^+.

Discussion

Root to shoot signaling of nutrient availability in the soil is critical for dynamic regulation of plant growth, which is of utmost importance for crop productivity under various environmental conditions. Potassium availability, as well as signaling of K^+ status to cells in the shoot, are important regulators of plant growth, development and tolerance to salt stress. Here, we analyzed the effects of blockage of K^+ channels in root cells on growth and photosynthesis of barley seedlings, using a specific blocker of K^+ transport, TEA^+. Tetraethylammonium ions are not toxic for cells in concentrations ranging from 0.5 to 50 mM (Touati *et al.* 2015). However, when introduced into soil, TEA^+ can cause some phytotoxic effects, similar to many other quaternary ammonium salt (QAS) pollutants (Pawlowska and Biczak 2016). Pawlowska and Biczak (2016) studied effects of TEA^+ in concentrations from 1 mg kg^{-1}(dry soil) to 5,000 mg kg^{-1}(dry soil) on seedlings of barley and radish

for 14 d and found that barley was much more susceptible to the phytotoxic effect of TEA^+ than radish. Seedlings exposed to TEA^+ displayed oxidative stress and growth inhibition, loss of chloroplast pigments, and damage of the photosynthetic apparatus. Neither of these symptoms was observed in this study.

The ability to regulate K^+ concentrations in both roots and shoots is related to salt tolerance in crops (reviewed by Wu *et al.* 2018). We used two barley varieties, Donetskiy and Donaria, which differed in their salt tolerance, as well as a Chl *b*-less mutant of Donaria, *clo-f2*. While TEA^+ slightly reduced germination rate and growth of Donetskiy, and had no effect on Donaria and *clo-f2*, 150 mM NaCl strongly reduced germination rate and growth of Donetskiy and *clo-f2*, especially when combined with TEA^+, and Donaria could hardly survive these treatments (data not shown). Unexpectedly, the *clo-f2* mutant showed a remarkably high germination rate and growth in the presence of NaCl, alone or combined with TEA^+, which was similar to, or even exceeded, that in salt-tolerant Donetskiy. No differences in K^+ contents in the shoots of these genotypes were observed here.

In barley seedlings grown on 1× Hoagland's reference medium, *i.e.*, in the presence of 5 mM K^+ and 6 mM Ca^{2+}, the most prominent response to TEA^+ was an increase in root growth and in the root/shoot ratio (Fig. 1). This is in marked contrast to what is known for plants experiencing K^+ deficiency. Potassium deficiency causes a pronounced decrease in root elongation and in the root/shoot ratio due to retardation of phloem transport (Hermans *et al.* 2006). An increase in biomass allocation to the roots of barley seedlings observed in our study indicates that phloem transport was not limited but rather increased. An increase in root growth and in the root/shoot ratio is a typical response to nitrogen limitation or water deficit (Hermans *et al.* 2006, Bogeat-Triboulot *et al.* 2007, Durand *et al.* 2016). As K^+ is the major counter-ion for nitrate transport from root to shoot, the reduction of K^+ uptake by TEA^+ might result in a reduction of both potassium and nitrate transport to shoots; this, however, was not the case (Fig. 2*B*). Furthermore, no signs of N starvation (*e.g.*, chlorosis) were observed. Alternatively, the exposure of barley seedlings to TEA^+ could have caused water deficit due to the decrease of root K^+ concentrations as well as a drop in the hydraulic conductivity of the plasma membrane of root cells (Liu *et al.* 2006, Touati *et al.* 2015). Consistent with the latter suggestion, the osmolality of root sap significantly increased, while root K^+ concentrations decreased, upon application of TEA^+ (Fig. 2*A*). Hence, we propose that the exposure of barley seedlings to TEA^+ induced a mild water deficit in roots due to reduced K^+ contents there (Fig. 2*C*), which acted as a signal to activate root growth, and thus increased the sink strength of the roots. This further led to a decrease in shoot growth combined with an increase of root elongation and of biomass allocation to roots.

The analysis of photosynthetic performance further confirmed this hypothesis. Previous studies of OJIP kinetics and JIP-tests in barley seedlings subjected to PEG-induced water deficit showed that increased osmotic stress led to a decrease in root growth and PI_{ABS} and to the appearance of positive K-bands indicative of PSII donor side limitation (Oukarroum *et al.* 2006). Similarly, field-grown barley plants experiencing water shortage displayed distinct positive K-bands and a decrease in PI_{ABS} which correlated with the level of drought susceptibility (Oukarroum *et al.* 2007). The PI_{ABS} combines three main functional steps of a PSII reaction center complex – absorption of light energy, trapping of excitation energy, and its conversion to electron transport – and has been established as the most sensitive parameter indicative of PSII performance to be used for the quantification of plant stress tolerance (Stirbet *et al.* 2018). In our experiments, PI_{ABS} increased significantly in response to the seedlings' exposure to TEA^+ in all genotypes. Moreover, $\varphi_{P0} = F_V/F_M$ increased significantly as well (Table 1). For Donetskiy, even accumulation of Chl *a* was observed which indicates an increase in RC numbers. Altogether, in this study, photosynthetic performance increased in response to TEA^+ treatment while shoot growth decreased, indicating the need to increase biomass allocation to roots to improve soil exploration in order to alleviate the K^+ deficit (Fig. 1).

While there are no reports of TEA^+ translocation in the xylem from roots to shoots, TEA^+ can be transported across the plasma membrane *via* NSCCs (Demidchik and Tester 2002), and can potentially accumulate in plant tissues. If this was the case, accumulation of TEA^+ would be expected to inhibit the function of K^+ channels as well as the expression of K^+ channel-encoding genes in photosynthesizing tissues. In chloroplasts, K^+ is critically important for the transport of H^+ into and out of thylakoid lumen (Pottosin and Shabala 2016); moreover, in plants lacking chloroplast K^+ transport systems, both establishment and relaxation of NPQ during the switch between high and low light are significantly retarded (Carrarretto *et al.* 2013, Dukic *et al.* 2019). However, the opposite was observed in this study: on medium with TEA^+, the rise of NPQ at high light, as well as its relaxation upon switching from high to low light, occurred significantly faster than on reference medium (Fig. 4*A*). Furthermore, no effects on NPQ were observed in leaves that had been fed TEA^+ *via* the transpiration stream for 10 h (Fig. 4*B*). These results strongly suggest that the changes in photosynthetic performance observed in whole seedlings were not caused by uptake and root-to-shoot translocation of TEA^+.

Salt stress exerts a double influence on photosynthesis as it has two components, the ionic and the osmotic one (Kaňa and Govindjee 2016). Excess of Na^+ and Cl^- negatively affects both the numbers of active PSII reaction centers and their performance, leading to degradation of the oxygen-evolving complex (OEC) and to limitation of electron transport beyond Q_{A^-} as shown by Yusuf *et al.* (2010) and Çiçek *et al.* (2018). In the same studies, NaCl induced positive K-bands in salt-sensitive varieties. However, no such effects were observed in our study. Altogether, the effects of barley seedlings' exposure to NaCl or NaCl combined with TEA^+ resembled the effects of TEA^+ alone in that root/shoot ratio increased relative to controls, and that photosynthetic performance per active PSII RC increased, combined with a decrease of

the proportion of the active PSII RC (although the latter effect was stronger under salt stress more than under TEA^+ treatment). We propose that under the experimental conditions used here, the effect of osmotic stress caused by NaCl was stronger than the negative influence of Na^+ or Cl^-, leading to increased biomass allocation to roots, while dramatically reducing seedling growth as compared to the control (Fig. 1). Another possible explanation is that Na^+ has an inhibitory effect on expression levels of genes encoding K^+ channels such as AKT1 (*e.g.*, Fuchs *et al.* 2005), which would mimic some effects of TEA^+.

An ameliorative effect of Ca^{2+} on salt stress is well established for shoots and roots (Demidchik and Tester 2002, Shabala *et al.* 2005, 2006). When barley seedlings were exposed to TEA^+, NaCl or their combination using 2× Hoagland's as reference medium, *i.e.*, upon doubling of Ca^{2+} and K^+ concentrations in the growth medium, the plants' responses changed. There was no increase in root elongation in seedlings exposed to TEA^+, and the increase in the root/shoot ratio was less pronounced for all genotypes and experimental conditions. Nevertheless, an increase in PI_{ABS} and φ_{P0} was observed in most cases (Table 2). In this series of experiments, the increase of K^+ concentrations in the growth medium seemed to attenuate the effect of TEA^+ while the increase in Ca^{2+} seemed to ameliorate the effects of NaCl stress.

The amplitudes of the K-bands observed in this study in all experiments increased in the following order: 2× Hoagland's + TEA^+ < 1× Hoagland's + TEA^+ < 2× Hoagland's + NaCl < 1× Hoagland's + NaCl ≃ 1× Hoagland's + NaCl +TEA^+ ≃ 2× Hoagland's + NaCl + TEA^+ (Fig. 3*C*). This order reflects more or less the increasing levels of stress experienced by seedlings: either inhibition of K^+/water uptake by TEA^+, or salt stress. Notably, an increase in stress levels was not accompanied by an increasing limitation of the donor side of PSII (which would appear as positive K-bands) but rather by faster electron transport within PSII (negative K-bands), an effect that can be partially explained by the stress-tolerance of barley. Altogether, the data show that JIP-tests and fast Chl *a* fluorescence transient kinetics can be used to detect the very first symptoms of signaling events indicating developing stress, long before the damage occurs

Xylem and phloem are the most suitable conduits for communication between roots and shoots. Inhibition of K^+ channels located in the xylem, like SKOR channels, is likely to cause changes in the membrane potential of the stele, similar to the effects exerted on these channels by abscisic acid (ABA) which is involved in both drought and salt stress signaling (Roberts and Snowman 2000, Zhu 2016). Recent results show that water movement and K^+ homeostasis are coregulated at different levels (Sustr *et al.* 2019). ABA is known to affect K^+ channel function as well as membrane potential in both guard cells and in the stelar parenchyma (Roberts and Snowman 2000, Jezek and Blatt 2017). A sensor function of plasma membrane transporters has been established for a nitrate transporter (Ho *et al.* 2009) and suggested for AKT1 and GORK (Ache *et al.* 2000, Li *et al.* 2017). Thus, it is tempting to speculate that K^+ channels could signal K^+ supply by affecting the stelar membrane potential directly, leading to the integration of drought and salt stress as well as K^+ signaling from root to shoot, regulating photosynthesis to meet changing root demands.

Conclusions: In contrast with previously reported responses to K^+ deficiency, TEA^+ inhibited shoot growth of barley seedlings while inducing an increase in the root/shoot fresh mass ratio indicating mild water deficit. Furthermore, while reducing shoot growth, TEA^+ increased photosynthetic performance and caused negative K-bands in OJIP kinetics. Similar negative K-bands were also induced in the presence of NaCl. Both TEA^+ and NaCl caused an increase in PI_{ABS}. These effects are interpreted as effects of root to shoot signaling communicating increased sink strength to the leaf photosynthetic machinery before deteriorative effects on photosynthesis could commence. Altogether, analysis of the state of the photosynthetic machinery using chlorophyll *a* transients monitored *via* JIP-tests allowed the detection of early signaling events prior to any deteriorative effects.

References

Ache P., Becker D., Ivashikina N. *et al.*: GORK, a delayed outward rectifier expressed in guard cells of *Arabidopsis thaliana*, is a K^+-selective, K^+-sensing ion channel. – FEBS Lett. **486**: 93-98, 2000.

Ball M.C., Chow W.S., Anderson J.M.: Salinity-induced potassium deficiency causes loss of functional photosystem II in leaves of the grey mangrove, *Avicennia marina,* through depletion of the atrazine-binding polypeptide. – Aust. J. Plant Physiol. **14**: 351-361, 1987.

Bogeat-Triboulot M.B., Brosché M., Renaut J. *et al.*: Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in *Populus euphratica*, a poplar growing in arid regions. – Plant Physiol. **143**: 876-892, 2007.

Carrarretto L., Formentin E., Teardo E. *et al.*: A thylakoid-located two-pore K^+ channel controls photosynthetic light utilization in plants. – Science **342**: 114-118, 2013.

Chow W.S., Ball M.C., Anderson J.M.: Growth and photosynthetic response of spinach to salinity: Implications of K^+ nutrition for salt tolerance. – Aust. J. Plant Physiol **17**: 563-578, 1990.

Çiçek N., Oukarroum A., Strasser R.J., Schansker G.: Salt stress effects on the photosynthetic electron transport chain in two chickpea lines differing in their salt stress tolerance. – Photosynth. Res. **136**: 291-301, 2018.

Demidchik V.: Mechanisms and physiological roles of K^+ efflux from root cells. – J. Plant Physiol. **171**: 696-707, 2014.

Demidchik V., Cuin T.A., Svistunenko D. *et al.*: *Arabidopsis* root K^+-efflux conductance activated by hydroxyl radicals: single-channel properties, genetic basis and involvement in stress-induced cell death. – J. Cell Sci. **123**: 1468-1479, 2010.

Demidchik V., Davenport R.J., Tester M.A.: Nonselective cation channels in plants. – Annu. Rev. Plant Biol. **53**: 67-107, 2002.

Demidchik V., Shabala S.N., Coutts K.B. *et al.*: Free oxygen radicals regulate plasma membrane Ca^{2+}-and K^+-permeable channels in plant root cells. – J. Cell Sci. **116**: 81-88, 2003.

Demidchik V., Tester M.A.: Sodium fluxes through nonselective cation channels in the plasma membrane of protoplasts from *Arabidopsis* roots. – Plant Physiol. **128**: 379-387, 2002.

Demidchik V., Tyutereva E.V., Voitsekhovskaja O.V.: The role of ion disequilibrium in induction of root cell death and autophagy by environmental stresses. – Funct. Plant Biol. **45**: 28-46, 2018.

Dreyer I., Gomez-Porras J. L., Riedelsberger J.: The potassium battery: a mobile energy source for transport processes in plant vascular tissues. – New Phytol. **216**: 1049-1053, 2017.

Dukic E., Herdean A., Cheregi O. *et al.*: K^+ and Cl^- channels/transporters independently fine-tune photosynthesis in plants. – Sci. Rep.-UK **9**: 8639, 2019.

Durand M., Porcheron B., Hennion N. *et al.*: Water deficit enhances C export to the roots in *Arabidopsis thaliana* plants with contribution of sucrose transporters in both shoot and roots. – Plant Physiol. **170**: 1460-1479, 2016.

Fuchs I., Stölzle S., Ivashikina N., Hedrich R.: Rice K^+ uptake channel OsAKT1 is sensitive to salt stress. – Planta **221**: 212-221, 2005.

Hermans C., Hammond J.P., White P.J., Verbruggen N: How do plants respond to nutrient shortage by biomass allocation? – Trends Plant Sci. **11**: 610-617, 2006.

Ho C.H., Lin S.H., Hu H.C., Tsay Y.F.: CHL1 functions as a nitrate sensor in plants. – Cell **138**: 1184-1194, 2009.

Hoagland D.R., Arnon D.I.: The Water-Culture Method for Growing Plants without Soil. Pp. 32. The College of Agriculture, University of California, Berkeley 1950.

Ivashikina N., Becker D., Ache P. *et al.*: K^+ channel profile and electrical properties of *Arabidopsis* root hairs. – FEBS Lett. **508**: 463-469, 2001.

Jezek M., Blatt M.R.: The membrane transport system of the guard cell and its integration for stomatal dynamics. – Plant Physiol. **174**: 487-519, 2017.

Kalaji H.M., Oukarroum A., Alexandrov V. *et al.*: Identification of nutrient deficiency in maize and tomato plants by *in vivo* chlorophyll *a* fluorescence measurements. – Plant Physiol. Bioch. **81**: 16-25, 2014.

Kalaji H.M., Rastogi A., Živčák 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.

Kaňa R., Govindjee: Role of ions in the regulation of light harvesting. – Front. Plant Sci. **7**: 1849, 2016.

Leigh R.A., Wyn Jones R.G.: A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. – New Phytol. **97**: 1-13, 1984.

Li J., Wu W.-H., Wang Y.: Potassium channel AKT1 is involved in the auxin-mediated root growth inhibition in *Arabidopsis* response to low K^+. – J. Integr. Plant Biol. **59**: 895-909, 2017.

Lichtenthaler H.K, Wellburn A.R.: Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. – Biochem. Soc. T. **11**: 591-592, 1983.

Liu H.-Y., Sun W.-N., Tang Z.-C.: Co-regulation of water channels and potassium channels in rice. – Physiol. Plantarum **128**: 58-69, 2006.

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.

Oukarroum A., El Madidi S., Strasser R.J.: Drought stress induced in barley cultivars (*Hordeum vulgare* L.) by polyethylene glycol, probed by germination, root length and chlorophyll *a* fluorescence rise (OJIP). – Arch. Sci. Genève **59**: 65-74, 2006.

Pawlowska B., Biczak R.: Evaluation of the effect of tetraethylammonium bromide and chloride on the growth and development of terrestrial plants. – Chemosphere **149**: 24-33, 2016.

Pier P.A., Berkowitz G.A.: Modulation of water-stress effects on photosynthesis by altered leaf K^+. – Plant Physiol. **85**: 655-661, 1987.

Plyusnina T.Yu., Khruschev S.S., Riznichenko G.Yu. *et al.*: An analysis of the chlorophyll fluorescence transient by spectral multi-exponential approximation. – Biofizika **60**: 392-399, 2015.

Pottosin I., Shabala S.: Transport across chloroplast membranes: Optimizing photosynthesis for adverse environmental conditions. – Mol. Plant **9**: 356-370, 2016.

Rabadanova C.K., Tyutereva E.V., Mackievic V.S. *et al.*: Cellular and molecular mechanisms controlling autophagy: A perspective to improve plant stress resistance and crop productivity. – Agric. Biol. **53**: 881-896, 2018.

Roberts S.K., Snowman B.N.: The effects of ABA on channel-mediated K^+ transport across higher plant roots. – J. Exp. Bot. **51**: 1585-1594, 2000.

Shabala S.: Signalling by potassium: another second messenger to add to the list? – J. Exp. Bot. **68**: 4003-4007, 2017.

Shabala S., Cuin T.A.: Potassium transport and plant salt tolerance. – Physiol. Plantarum **133**: 651-669, 2008.

Shabala S., Demidchik V., Shabala L., Newman I.: Extracellular Ca^{2+} ameliorates NaCl-induced K^+ loss from *Arabidopsis* root and leaf cells by controlling plasma membrane K^+-permeable channels. – Plant Physiol. **141**: 1653-1665, 2006.

Shabala S., Shabala L., van Volkenburgh E. *et al.*: Effect of divalent cations on ion fluxes and leaf photochemistry in salinized barley leaves. – J. Exp. Bot. **56**: 1369-1378, 2005.

Stirbet A., Lazár D., Kromdijk J., Govindjee: Chlorophyll *a* fluorescence induction: Can just a one-second measurement be used to quantify abiotic stress responses? – Photosynthetica **56**: 86-104, 2018.

Strasser R.J., Srivastava A., Tsimilli-Michael M.: The fluorescence transient as a tool to characterize and screen photosynthetic samples. – In: Yunus M., Pathre U., Mohanty P. (ed.): Probing Photosynthesis: Mechanisms, Regulation and Adaptation. Pp. 445-483. Taylor & Francis, London 2000.

Strasser R.J., Tsimilli-Michael M., Srivastava A.: Analysis of the chlorophyll *a* fluorescence transient. – In: Papageorgiou G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration. Pp. 321-362. Springer, Dordrecht 2004.

Sustr M., Soukup A., Tylova E.: Potassium in root growth and development. – Plants **86**: 435, 2019.

Touati M., Knipfer T., Visnovitz T. *et al.*: Limitation of cell elongation in barley (*Hordeum vulgare* L.) leaves through mechanical and tissue-hydraulic properties. – Plant Cell Physiol. **56**: 1364-1373, 2015.

Udovenko G.V., Volkova A.M.: [Determination of salinity tolerance in crops at the early developmental stage using a complex of growth characteristics (guidelines).] Pp. 15. N.I. Vavilov Institute, St. Petersburg 1993. [In Russian]

Wu H., Zhang X., Giraldo J.P., Shabala S.: It is not all about sodium: Revealing tissue specificity and signalling roles of potassium in plant responses to salt stress. – Plant Soil **431**: 1-17, 2018.

Yusuf M.A., Kumar D., Rajwanshi R. *et al.*: Overexpression of γ-tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: Physiological and chlorophyll *a* fluorescence measurements. – BBA-

Bioenergetics **1797**: 1428-1438, 2010.
Zhu J.-K.: Abiotic stress signaling and responses in plants. – Cell **167**: 313-324, 2016.
Zörb C., Senbayram M., Peiter E.: Potassium in agriculture – Status and perspectives. – J. Plant Physiol. **171**: 656-669, 2014.