Potassium deficiency impact on the photosynthetic apparatus efficiency of radish

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Abstract

Potassium is an essential nutrient for plants and its limited resources impose the improvement of the fertilizer-use efficiency. To address this issue, it is important to understand its roles in the photosynthesis and to provide a quick, noninvasive method for diagnosing its deficiency in plants. The impact of potassium deficiency on the photochemical phase of photosynthesis has not been well understood. In this work, we present the K^+-deficiency effect on gas exchange, chlorophyll content, and electron transport chain in two hybrid cultivars of radish. Our results showed that one of the cultivars proved to be tolerant and maintained the net photosynthetic rate at the level of the control plants. In both cultivars, the effect on the photochemical phase of photosynthesis was observed. We suspect that low K^+ availability impaired the balance of the H^+ influx to thylakoid lumen which increased its acidification and triggered the downregulation mechanism leading to the rearrangement of PSII complexes and an increase in energy dissipation.

Introduction

Potassium is one of the key nutrients the availability of which strongly determines plant growth and development. The deficiency of this element in arable soils is common.

At the same time, limited resources of potassium fertilizers are now forcing us to improve fertilizer-use efficiency. To address this issue, we need to understand the effect of low K^+ availability for plants. The possibility of early diagnosis of potassium deficiency in plants would also

Highlights

- P_N reduction under K deficiency in radish was not caused by stomatal limitation
- K deficiency affected the thermal phase (2–30 ms) of chlorophyll *a* fluorescence transient
- K deficiency affected the PSII connectivity and the redox state of PQ pool

help improve the fertilizer-use efficiency. Until now, a stress caused by a deficiency of various macronutrients was most often diagnosed based on the visual symptoms, that is when the deficiency of a given nutrient has already caused damage to the plant. Moreover, to obtain reliable information about a plant nutrient status, an analysis of the chemical composition of plant tissue is required. This method is both expensive and time-consuming. Therefore, there is a need for a method that allows for a quick and noninvasive diagnosis of nutrient deficiency in plants, including potassium.

Understanding the exact role of potassium in the plant, including photosynthesis, is the basis for answering these issues. The availability of this component has been positively correlated with photosynthetic productivity of plants. However, the impact of deficiencies of this key nutrient on the course and efficiency of the photochemical phase of photosynthesis has not been well understood.

Potassium, along with nitrogen and phosphorus, is one of the most important nutrients; their availability regulates plant growth and development. However, unlike nitrogen and phosphorus, potassium is not a constituent of organic compounds. In plants, it occurs almost exclusively in ionic form and is one of the most mobile elements. In leaf cells, potassium is mainly found in mitochondria, chloroplasts, and vacuoles (Mengel 2006). Potassium ions activate the action of many enzymes: transferases, oxidoreductases (mainly dehydrogenases), synthetases, kinases, nitrate reductase, enzymes of carbohydrate and protein metabolism, as well as membrane ATPase. The specific functions of potassium ions include regulation of plant water management, transpiration, control of stomata movement, regulation of membrane permeability, and cell osmoregulation. Potassium ions are involved in intercellular and interorgan transport, peptide synthesis, and stress response. The balance between K^+ and Na^+ ions regulates the function of chloroplasts, and plants with potassium deficiency show greater sensitivity to light and peroxide overproduction (Pottosin and Shabala 2016). Studies on photosynthetic efficiency in conditions of potassium deficiency have shown a reduction in chlorophyll (Chl) content, net photosynthesis efficiency, and stomatal conductance, inhibition of photosynthetic phosphorylation, reduction of photochemical fluorescence quenching (q_P), and electron transport rate through PSII (ETR) as well as a reduction in the activity and concentration of Rubisco in wheat, rice, corn, potato, sweet potato, cotton, and soybean (Osaki *et al.* 1993, Zhao *et al.* 2001, Jia *et al.* 2008, Wang *et al.* 2015, Liu *et al.* 2017). Degl'Innocenti *et al.* (2009) in their research on barley proved that potassium deficiency not only negatively affected CO_2 assimilation, but also increased the negative effects of salinity on photosynthesis. At the same time, some researchers reported differences between cultivars of a given species in response to potassium deficiency. Cultivars with high tolerance to this type of stress reduced photosynthetic productivity to a lesser extent (Jia *et al.* 2008, Zhao *et al.* 2016, Liu *et al.* 2017). Liu *et al.* (2017) in their research on sweet potato – a species with a high demand for potassium – did not report significant changes in the parameters of the JIP-test in a

cultivar with an increased tolerance. However, according to Jákli *et al.* (2017) sunflower potassium deficiency did not directly affect either the PSII quantum yield or Rubisco activity *in vivo*, and CO_2 assimilation was inhibited mainly by reducing stomata conductivity. It has not yet been determined whether sources of limited CO_2 assimilation in conditions of potassium deficiency should be sought in photochemical phase disturbances, inhibition of the biochemical phase, or lower CO_2 availability resulting from the limitation of gas exchange by stomata.

One of the methods used in the study of the light-dependent phase of photosynthesis is the analysis of direct Chl *a* fluorescence. Although fluorescence is a small part of the energy emitted from the photosynthetic apparatus, its analysis allows describing both the functioning and certain structural features of the photosynthetic apparatus (Strasser *et al.* 2004, Stirbet *et al.* 2014, Dąbrowski *et al.* 2016). Detailed fluorescence signal analysis, so-called JIP-test, enables for a noninvasive monitoring of the current functioning of primary photosynthesis reactions in various environmental conditions. The relationship between photosynthesis reactions and Chl fluorescence signals from the JIP-test are based on the theory of energy flow in thylakoid membranes (Strasser *et al.* 2004). Based on this theory, algebraic equations have been created describing the balance between the inflow and outflow of total energy for analyzed photosynthetic pigment–protein complexes. These equations allow us to obtain information on the probability of the fate of absorbed energy and to characterize energetic communication between PSII components, describing all routes of this communication. Chl *a* fluorescence measurements and their analysis using the JIP-test are used in studies of plant responses to stress caused by various environmental factors (Strasser *et al.* 2004, Kalaji *et al.* 2014, Stirbet *et al.* 2018, Dąbrowski *et al.* 2019, Tsimilli-Michael 2020), including nutrient deficiencies (Goltsev *et al.* 2012, Aleksandrov *et al.* 2014, Živčák *et al.* 2014, Cetner *et al.* 2017, 2020; Horaczek *et al.* 2020, Samborska-Skutnik *et al.* 2020).

The study aimed to examine the efficiency of the photochemical phase of photosynthesis, determine the modification of its course and structural changes of pigment–protein complexes in radish (*Raphanus sativus* var. *sativus*) growing in conditions of potassium deficiency.

Materials and methods

Plant material and growth conditions: Two different hybrid cultivars of radish were chosen for the experiments: *Raphanus sativus* var. *sativus* 'Suntella F1' (Suntella) and 'Fluo HF1' (Fluo). Plants were grown in a phytotron under controlled conditions. Photoperiod was 14 h, day/night air temperature was 18/13°C, PPFD was 250 μmol(photon) m^{-2} s^{-1}, and 24-h average air humidity was approx. 50%. Radish was grown in hydroponics, because this method allows for a better control of the amount of nutrients supplied to plants. An innovative material was used as the substrate: polyethylene pellet (PE), which has a low ion absorption capacity as compared to commonly used mineral substrates. The media were developed based on

Hoagland's medium (Hoagland and Arnon 1950):

	Control solution	Experimental solution
Macronutrients [mmol L^{-1}]		
N (NO$_3$)	11.63	12.14
P (PO$_4$)	1.00	1.50
K	6.28	0.00
Ca	4.00	4.00
Mg	2.00	2.00
Na	1.13	1.14
S (SO$_4$)	3.13	0.14
Cl	1.00	4.00
Micronutrients [μmol L^{-1}]		
Fe	118.48	118.48
B	48.57	48.57
Cu	1.60	1.60
Zn	0.97	0.97
Mn	6.51	6.51
Mo	0.52	0.52

Tap water was used for seed germination and initial plant growth (8 d). After that, plants were grown on the control medium for 10 d until the first pair of true leaves developed. From this moment, the plants were exposed to a stressor; the first day of stress conditions is referred to as 1 DAT. Stress conditions were maintained until over 50% of plants developed symptoms of potassium deficiency, *i.e.*, chlorotic and necrotic spots, which were observed on 17 DAT. Then the control medium was restored to the plants (regeneration period, 8 d).

The fresh and dry mass of leaves as well as the content of selected mineral components were determined 7 times during plants' life cycle: on 1, 3, 6, 10, 15, 20, and 24 DAT. In order to obtain > 1 g of dry mass for mineral composition analyses, 3 to 27 plants were randomly harvested; after removing roots, bulbs, and cotyledons, leaves were weighed, dried, and then weighed again. The chemical composition analyses were carried out on collective samples of 3–27 randomly selected plants.

Potassium content was determined using atomic emission spectrometry (ICP-AES, *Thermo Scientific iCAP 6000*, *Thermo Fisher Scientific Inc.*, USA), after mineralization of plant material in 65% HNO$_3$ and 70% HClO$_4$ (HNO$_3$:HClO$_4$ = 4:1).

***In vivo* Chl content** was determined using *Dualex Scientific+™ Polyphenol & Chlorophyll-Meter* (*Force-A*, France) several times during the vegetation period, at 2–4-d intervals. Measurements were carried out on 2–3 fully developed leaves of nine plants, selected at random and marked before stress conditions were introduced.

Plant gas-exchange parameters: Net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) and intercellular concentration of CO$_2$ (C_i) were determined at approximately 7-d intervals using *LCpro+* infrared gas analyzer (*ADC BioScientific Ltd.*, UK). Gas exchange was determined in ambient phytotron conditions: day/night air temperature of 18/13°C, PPFD of 250 μmol(photon) m^{-2} s^{-1}, and CO$_2$ concentration around 500 ppm. Measurements were performed on four fully developed leaves of randomly selected plants.

Chl *a* fluorescence intensity was measured *in vivo* using a *HandyPEA* fluorimeter (*Hansatech Instruments Ltd.*, Norfolk, UK). Measurements were carried out after 30-min adaptation to darkness, on the same leaves of 36 plants selected at random and marked before stress conditions were introduced. Measurement data was used to calculate the JIP-test parameters. The meanings of individual parameters and mathematical formulas used in their calculations are presented in the Appendix.

Statistical analysis: The experimental data were statistically analyzed using one- and two-factor analysis of variance (*ANOVA*). Significance of treatment effects was analyzed by the *Tukey*'s test at *P*-value<0.05. The main components analysis (PCA) was performed to evaluate the relationship between the analyzed features and to characterize multivariate differences between them. Calculations were performed using *Microsoft Excel* (*Microsoft*, USA), *StatPlus:mac* (*AnalystSoft Inc.*, USA), and *STATISTICA 13.1* (*StatSoft*, Tulsa, USA).

Results

Potassium content in leaves: The optimal potassium content in radish leaves is > 2.0% (Sanchez *et al.* 1991). In an experiment on radish growing in potassium deficiency, a reduction of this element to 1.5% compared to 2.2% in the control was observed as early as on 1 DAT in case of Suntella (Fig. 1*A*), while in Fluo the difference was observed on 3 DAT: 1.9 compared to 2.7% in the control (Fig. 1*B*). The increase in K$^+$ concentration in K-deficient Suntella to 1.9% on 3 DAT may result from the translocation of K$^+$ from cotyledons, which were excluded from the analysis of chemical composition. The low K$^+$ content in the leaves of both cultivars was maintained throughout the entire stress period, reaching the lowest level on 15 DAT (0.3 and 0.6% in Suntella and Fluo cultivars, respectively). After restoring the potassium-containing medium (recovery period), the K$^+$ content in both cultivars increased to the level observed in the control plants. Radish for a long time did not show symptoms considered typical for potassium deficiency. The wrapping of the edges of the leaf blades and the appearance of necrotic spots between leaf veins on the lower leaves were observed only on 10 DAT. Specific symptoms in approx. 50% of plants appeared on 14 DAT and were more pronounced in the case of the Suntella cultivar.

Dry mass of leaves: No significant changes in dry mass were observed in potassium-deficient and control plants (Fig. 1*C,D*). A decrease in dry mass compared to the control sample was observed only on 20 DAT, *i.e.*, during the regeneration period. The reduction in plant mass was

partly due to the drying and falling off of the old (lower) leaves, especially in the case of the Suntella cultivar.

Chlorophyll content: The Chl content in leaves of plants exposed to potassium deficiency differed between the two cultivars. In the case of the Suntella cultivar, the Chl content

remained at or exceeded the control value – a significantly higher value was recorded on 13 DAT (Fig. 2*A*). However, in the case of Fluo, the Chl content remained at the control level up to 13 DAT, while from 15 DAT it was decreasing; at the end of the growing season, the content of Chl in the leaves was lower compared to the control (Fig. 2*B*).
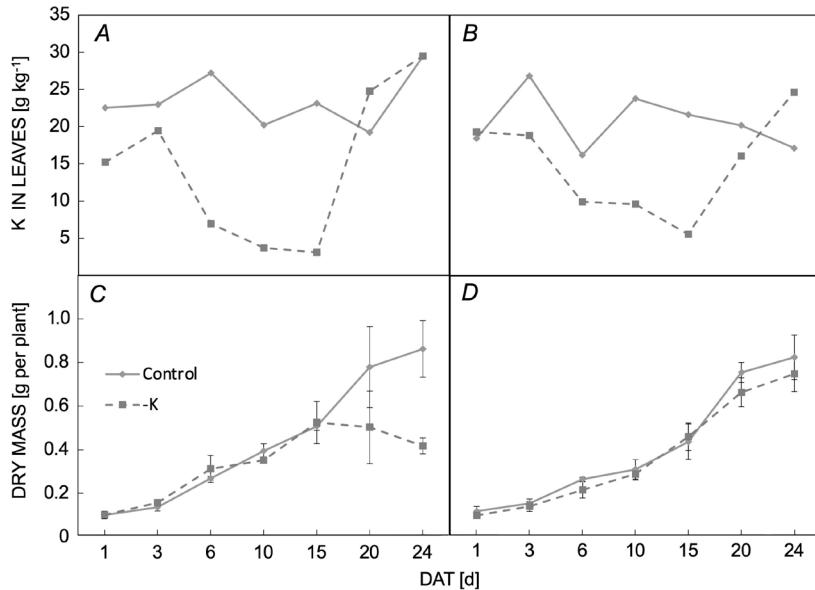
Fig. 1. Impact of potassium deficiency stress on K content in leaves (*A,B*) and dry mass per plant (*C,D*) of two radish cultivars: 'Suntella F1' (*A,C*) and 'Fluo HF1' (*B,D*). DAT – days after treatment. K content was evaluated for a pool sample of 3–9 plants. The mean ± SD for dry mass per plant was calculated from three repetitions.
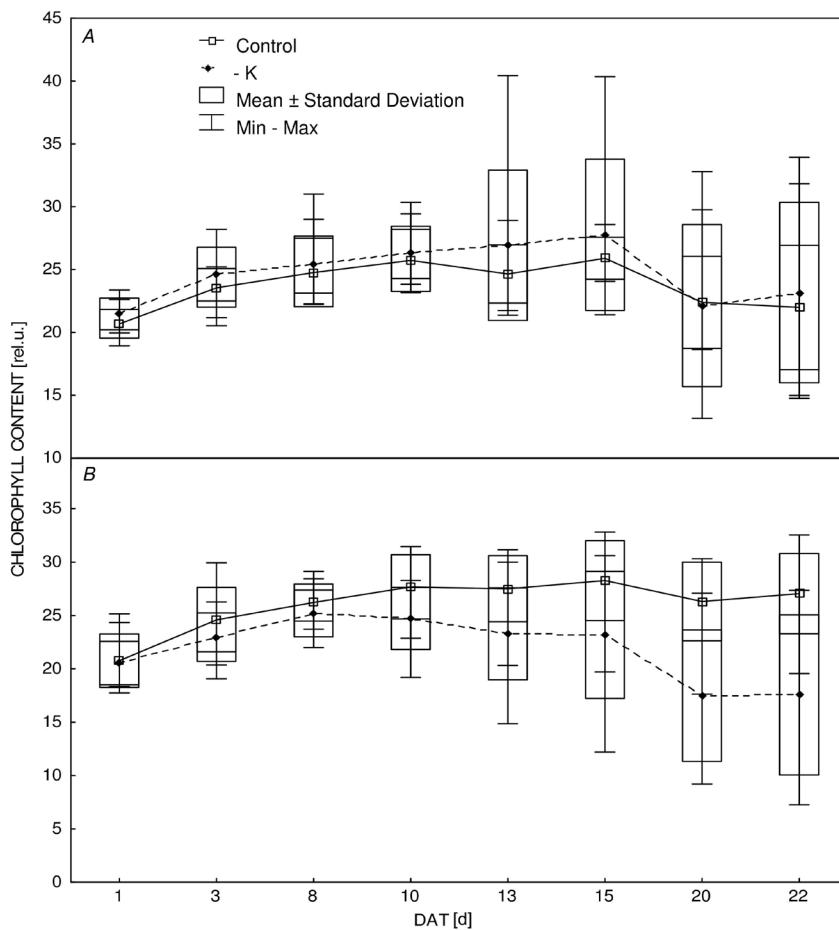

Fig. 2. Impact of potassium deficiency stress on chlorophyll content of two radish cultivars 'Suntella F1' (*A*) and 'Fluo HF1' (*B*). DAT – days after treatment. The mean ± SD was calculated from nine repetitions.

Gas-exchange parameters: On the contrary, the net photosynthetic rate (P_N) decreased only in case of the Suntella cultivar (Fig. 3*A,B*), while Fluo was able to maintain P_N at the level of control plants even after 15 DAT, when its Chl content decreased. The transpiration rate (E) in both cultivars remained unchanged as compared to the control sample throughout the growing season (Fig. 3*C,D*). Moreover, no significant reduction in stomatal conductance (g_s) was observed (Fig. 3*E,F*). On the contrary, at the beginning of stress, on 3 DAT, more than a two-fold increase in the value of this parameter was noted.

The OJIP curve: Chl *a* fluorescence analysis showed changes in the shape of the OJIP curve in both cultivars

subjected to potassium deficiency stress (Fig. 4). The change in the shape of the curve was more pronounced in young (upper) leaves than that in old (lower) ones (Fig. 4*C,D*). This may indicate that light phase disorders affected young leaves to a greater extent, despite the high K⁺ mobility and the ease of their translocation to developing tissues. Additionally, the rise in fluorescence intensity was higher in the case of Suntella, which also showed a decrease in P_N. Increased fluorescence intensity was observed mainly in the J–I phase (2–30 ms), *i.e.*, at the beginning of the so-called thermal phase. The phase was named so because of its sensitivity to temperature, which results from the initiation of the electron exchange between PQH_2/PQ and Q_B and then cytochrome (Cyt) b_6f
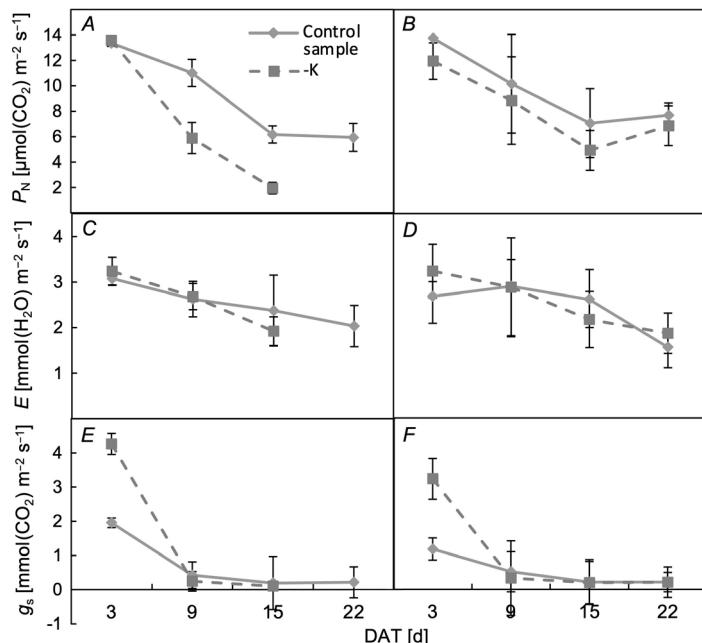

Fig. 3. Impact of potassium deficiency stress on net photosynthesis rate, P_N (*A,B*), transpiration rate, E (*B,C*), and stomatal conductance, g_s (*E,F*) of two radish cultivars: 'Suntella F1' (*A,C,E*) and 'Fluo HF1' (*B,D,F*). DAT – days after treatment. The mean ± SD was calculated from three repetitions.
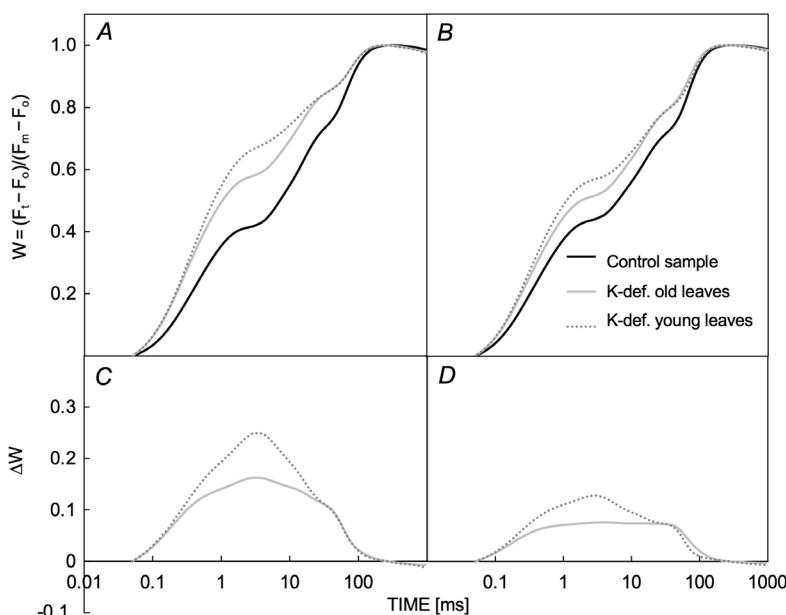
Fig. 4. Changes in the shape of relative chlorophyll *a* fluorescence fast induction curves revealed from dark-adapted leaves of different age (old, bottom leaves, and young, upper leaves) of K-deficient *Raphanus sativus* var. *sativus* cultivars 'Suntella F1' (*A,C*) and 'Fluo HF1' (*B,D*) on 15 DAT. The curves were double normalized [W = (F_t – F_o)/(F_m – F_o), where F_o = $F_{0.05ms}$, F_m = maximal fluorescence value, and F_t = fluorescence value at given time t; panels *A,B*] and presented as difference transients ΔW between stressed and control samples (panels *C,D*). Mean values were derived from 36 measurements per treatment.

(Stirbet and Govindjee 2012). While the course of the photochemical phase (O–J) is affected mainly by PSII activity, the thermal phase is additionally affected by PSI activity. According to Stirbet and Govindjee (2012), in the J–I–P phase, the PSII reaction centers are still gradually closing, with the parallel reduction of the plastoquinone pool.

The JIP-test: The results of the JIP-test are presented in Table 1. A decrease in the quantum yield of primary PSII photochemistry (ϕ_{Po}) revealed disorders in PSII.

We also observed changes in parameters connected with the mechanisms protecting PSII against photoinhibition, *i.e.*, disconnection of LHCII complexes (increase in F_o; with the exception of old leaves of K-deficient Suntella plants), reduction in the number of active reaction centers (RC/ABS) and the number of Q_A reduction events (N) followed by an increase in the net rate of the RCs' closure (M_0). The density of active RC PSII per cross-section (RC/CS_0) remained at the level of the control sample in Fluo, but it decreased in the case of Suntella. This is probably due to Suntella's high Chl content, and thus a

Table 1. Impact of potassium deficiency on the values of the JIP-test parameters of two radish cultivars 'Suntella F1' and 'Fluo HF1' on 15 DAT. The mean ± SE was calculated from 20–36 observations. Data were analyzed using two-factor *ANOVA*. Significant differences were marked * ($\alpha = 0.05$), ns – no difference.

Parameter	Old leaves		Young leaves	
	Control sample	–K	Control sample	–K
'Suntella F1'				
$F_o = F_{0.05\ ms}$	678 ± 55	832 ± 67^{ns}	638 ± 19	$909 \pm 48^{*}$
F_m	$3{,}222 \pm 41$	$2{,}488 \pm 162^{*}$	$3{,}304 \pm 40$	$2{,}222 \pm 137^{*}$
T_{Fm}	304.444 ± 9.228	275.000 ± 29.437^{ns}	335.278 ± 12.094	302.000 ± 36.256^{ns}
M_0	0.703 ± 0.036	$1.194 \pm 0.079^{*}$	0.575 ± 0.018	$1.198 \pm 0.078^{*}$
N	20.103 ± 0.946	$17.040 \pm 0.982^{*}$	21.791 ± 0.344	$16.757 \pm 0.506^{*}$
ϕ_{Po}	0.793 ± 0.013	$0.627 \pm 0.038^{*}$	0.807 ± 0.005	$0.561 \pm 0.032^{*}$
ϕ_{Eo}	0.470 ± 0.012	$0.261 \pm 0.032^{*}$	0.508 ± 0.007	$0.198 \pm 0.026^{*}$
ϕ_{Ro}	0.187 ± 0.006	$0.099 \pm 0.010^{*}$	0.256 ± 0.004	$0.088 \pm 0.009^{*}$
ψ_{Eo}	0.589 ± 0.008	$0.382 \pm 0.032^{*}$	0.629 ± 0.006	$0.331 \pm 0.028^{*}$
δ_{Ro}	0.405 ± 0.014	0.443 ± 0.038^{ns}	0.505 ± 0.006	0.484 ± 0.028^{ns}
RC/ABS	0.484 ± 0.015	$0.335 \pm 0.023^{*}$	0.530 ± 0.010	$0.327 \pm 0.023^{*}$
TR_0/RC	1.686 ± 0.045	$1.907 \pm 0.036^{*}$	1.540 ± 0.026	$1.763 \pm 0.050^{*}$
ET_0/RC	0.983 ± 0.014	$0.713 \pm 0.053^{*}$	0.965 ± 0.014	$0.565 \pm 0.039^{*}$
RE_0/RC	0.398 ± 0.017	$0.285 \pm 0.014^{*}$	0.488 ± 0.010	$0.259 \pm 0.011^{*}$
PI_{abs}	3.138 ± 0.180	$0.874 \pm 0.214^{*}$	3.985 ± 0.177	$0.455 \pm 0.137^{*}$
PI_{tot}	2.138 ± 0.172	$0.677 \pm 0.204^{*}$	4.077 ± 0.200	$0.343 \pm 0.087^{*}$
RC/CS_0	303.276 ± 4.141	$262.038 \pm 15.604^{*}$	332.06 ± 2.908	$284.834 \pm 16.568^{*}$
'Fluo HF1'				
$F_o = F_{0.05\ ms}$	671 ± 42	$810 \pm 42^{*}$	679 ± 22	$884 \pm 37^{*}$
F_m	$3{,}137 \pm 42$	$2{,}796 \pm 101^{*}$	$3{,}234 \pm 39$	$2{,}745 \pm 85^{*}$
T_{Fm}	300.294 ± 8.647	$237.500 \pm 6.671^{*}$	305.833 ± 9.406	$241.250 \pm 9.287^{*}$
M_0	0.706 ± 0.044	$1.023 \pm 0.048^{*}$	0.677 ± 0.026	$1.099 \pm 0.061^{*}$
N	21.299 ± 0.731	$17.705 \pm 0.567^{*}$	21.160 ± 0.365	$18.819 \pm 0.479^{*}$
ϕ_{Po}	0.785 ± 0.014	$0.699 \pm 0.017^{*}$	0.791 ± 0.005	$0.666 \pm 0.018^{*}$
ϕ_{Eo}	0.466 ± 0.015	$0.337 \pm 0.019^{*}$	0.465 ± 0.010	$0.289 \pm 0.021^{*}$
ϕ_{Ro}	0.228 ± 0.006	$0.148 \pm 0.008^{*}$	0.242 ± 0.007	$0.141 \pm 0.009^{*}$
ψ_{Eo}	0.587 ± 0.012	$0.471 \pm 0.018^{*}$	0.587 ± 0.009	$0.421 \pm 0.022^{*}$
δ_{Ro}	0.502 ± 0.016	$0.447 \pm 0.014^{*}$	0.518 ± 0.008	0.509 ± 0.015^{ns}
RC/ABS	0.483 ± 0.016	$0.378 \pm 0.016^{*}$	0.493 ± 0.011	$0.371 \pm 0.018^{*}$
TR_0/RC	1.672 ± 0.044	$1.917 \pm 0.052^{*}$	1.623 ± 0.027	$1.868 \pm 0.054^{*}$
ET_0/RC	0.967 ± 0.014	0.894 ± 0.035^{ns}	0.945 ± 0.013	$0.769 \pm 0.035^{*}$
RE_0/RC	0.483 ± 0.014	$0.403 \pm 0.024^{*}$	0.490 ± 0.010	$0.381 \pm 0.015^{*}$
PI_{abs}	3.199 ± 0.238	$1.300 \pm 0.202^{*}$	2.984 ± 0.218	$1.006 \pm 0.213^{*}$
PI_{tot}	3.004 ± 0.214	$0.988 \pm 0.146^{*}$	3.330 ± 0.269	$0.927 \pm 0.192^{*}$
RC/CS_0	303.451 ± 4.321	290.133 ± 8.979^{ns}	327.830 ± 3.913	311.868 ± 9.191^{ns}

necessity of protection of the photosynthetic apparatus against photoinhibition by converting a greater amount of RC into so-called silent RCs, non-Q_A^--reducing RCs which dissipate the excessive energy (Strasser *et al.* 2004). Parameters which characterize quantum yields of electron transport (ϕ_{Eo}), electron transport until PSI electron acceptors (ϕ_{Ro}), as well as efficiency/probability with which an electron trapped in PSII RC is transferred beyond Q_A^- (ψ_{Eo}) were reduced to a greater extent, although the parameter δ_{Ro}, which describes efficiency/probability with which an electron from Q_B is transferred until PSI acceptors, decreased only in the case of old leaves of the the Fluo cultivar. Although trapped energy flux per RC (TR_0/RC) increased as compared to the control sample, the electron transport flux further than Q_A^- (ET_0/RC) and electron transport flux until PSI acceptors (RE_0/RC) were significantly lower. As a result, the performance indexes for energy conservation from photons absorbed by PSII antenna to the reduction of Q_B (PI_{abs}) and PSI acceptors (PI_{tot}) decreased.

The principal component analysis: The results of PCA analysis were shown in Fig. 5. The first component (PC1) explains 78.1% of the tested variation, the second (PC2) 14.0%. Potassium availability strongly affected the plant mass (FW, DM), values of the JIP-test parameters (RC/ABS, ET_0/RC, PI_{tot}, PI_{abs}, F_v/F_m) as well as the intensity of photosynthesis (P_N), transpiration (E), and potassium content in plants (K). These features were significantly positively correlated. Simultaneously, trapped energy flux per RC (TR_0/RC) was negatively correlated, whereas the Chl content in plants was not correlated with these characteristics.

Discussion

Two radish cultivars showed different reaction to potassium deficiency. The overall stress reaction was more pronounced in the case of the Suntella cultivar; its K^+ content decreased earlier and to a greater extent, plants developed more severe deficiency symptoms, net photosynthetic rate decreased, and more absorbed energy was reemitted as Chl *a* fluorescence. The only feature that remained unaffected in Suntella was its Chl content. At the same time, the Chl content in Fluo decreased significantly after 15 DAT which is around the time the deficiency symptoms were fully developed. A slight decrease in the Chl content at the end of the vegetation period in the case of Suntella was most likely due to the leaf senescence.

A similar observation, *i.e.*, significantly different Chl content in two maize cultivars under potassium deficiency was found by Zhao *et al.* (2016). In studies of Zhao *et al.* (2016), one of the cultivars showed a higher tolerance to potassium deficiency stress, which was manifested in the ability to maintain a high Chl content as well as higher P_N and yield of PSII primary photochemical reactions as compared to the sensitive cultivar. However, in our experiment with radish growing in conditions of potassium deficiency, the opposite results were observed. The cultivar which showed no decrease in Chl content proved to be the sensitive one and Chl content did not show a correlation with photosynthetic productivity (Fig. 5). These findings combined with the JIP-test results allow for a conclusion, that in the case of the Suntella cultivar a substantial amount of energy absorbed in light-harvesting complexes was dissipated due to employment of photoprotective mechanisms, including structural rearrangements of the
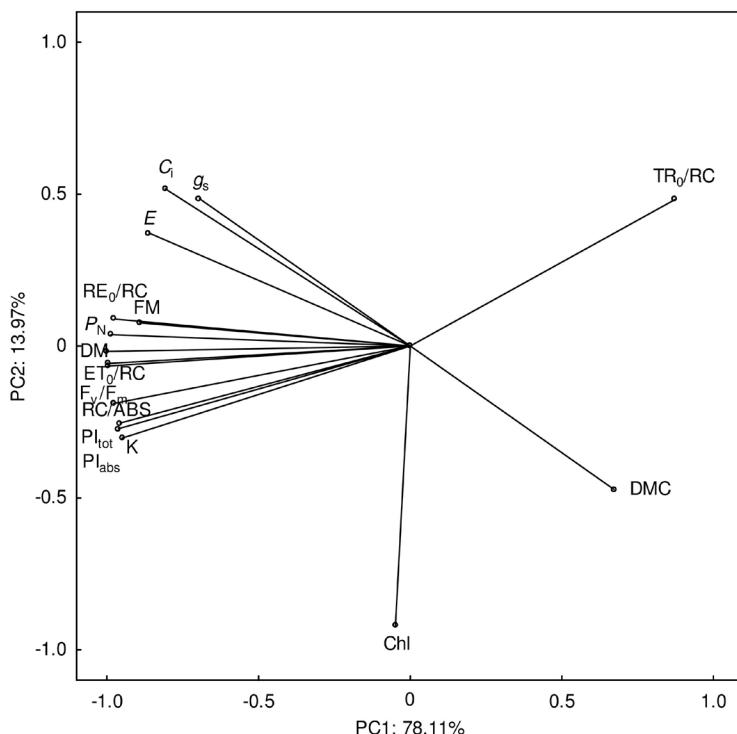

Fig. 5. Principal component analysis (PCA) of K-deficiency stress effect on photosynthetic activity in leaves of radish plants during vegetation period. PCA is based on combined data from fresh mass (FM), dry mass (DM), dry mass content (DMC), net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), chlorophyll content (Chl), and the JIP-test parameters (as described in Appendix) measured in leaves of radish cultivars 'Fluo HF1' and 'Suntella F1'. *The arrows* present vectors in PC coordinates, reflecting the influence of corresponding parameter on the calculated values of PC1 and PC2.

complexes (LHCII detachment) and increased number of silent RCs.

In their research on cotton and sunflower growing in potassium-deficient conditions, Bednarz *et al.* (1998) and Jákli *et al.* (2017) concluded that the main reason for the reduction of P_N of plants was stomatal limitation. On the contrary, Arquero *et al.* (2006) observed enhanced stomatal conductance in both well-irrigated and water-stressed olive trees and concluded that moderate K deficiency may impair the plant's ability to regulate stomatal closure. Stomatal closure impairment caused by a moderate K-deficiency stress could be the reason for the increase in stomatal conductance observed on 3 DAT in radish. K deficiency is also associated with decreased transpiration rates (Arquero *et al.* 2006). Although a crucial role of K^+ in cell osmoregulation, turgor maintenance, and stomatal function has been established, the complexity of stomata behavior under real-world conditions (Shabala 2003) makes difficult to draw clear conclusions on the relationship between potassium nutrition and plant water status and/or stomatal activity. Nevertheless, in the experiment on radish under K-deficiency conditions, neither transpiration rate nor stomatal conductance decreased. Moreover, we conclude that stomatal conductance was not among the factors limiting CO_2 assimilation in the case of the Suntella cultivar. Hence, we speculate that the mechanisms underlying the limitation of CO_2 accumulation may result from a decrease in Rubisco activity and/or disorders in photochemical phase of photosynthesis.

The analysis of Chl *a* fluorescence pointed out the beginning of the so-called thermal phase, from 2 to 30 ms, as a place where major disturbances occurred. Reported factors that affect the course of the thermal phase include the redox state of the plastoquinone pool, PSII connectivity, PSII and plastoquinone pool size heterogenity, as well as alternative electron transport pathways (Stirbet and Govindjee 2012). Our results suggest that K deficiency affected the PSII connectivity and the redox state of PQ pool in radish. Therefore, factors that influence PQ/PQH_2 exchange reactions, *i.e.*, pH at the reaction site, proton gradient, and electric potential across the thylakoid membrane, require special attention in this kind of stress. The transmembrane pH gradient is generated by the activity of plastoquinones at PSII acceptor side; on the other hand, the pH gradient plays additional role in the feedback downregulation of light harvesting and electron transport (Galvis *et al.* 2020). There are certain thresholds of proton concentration, above which the two processes of feedback downregulation are induced; below lumen pH of 6.8, the protonation of the thylakoid PsbS protein occurs, which leads to the rearrangement of PSII complexes and an increase in the thermal dissipation of absorbed energy, while below lumen pH of 6.5, PQH_2 oxidation at the Cyt b_6f complex is restrained (Galvis *et al.* 2020). In their research on phosphorus-deficient barley, Carstensen *et al.* (2018) reported that the mechanism of preventing PQH_2 oxidation by lumen acidification affected the Chl *a* fluorescence induction curve by flattening the I-step. The I-step, *i.e.*, the point of inflection of the curve at 30 ms, results from balancing the reduction of the plastoquinones on the acceptor side of PSII and their oxidation at the

Cyt b_6f complex; at this stage, PQH_2 oxidation reaches its maximum rate because the subsequent ETC carriers after Cyt b_6f remain oxidized (Schansker *et al.* 2005, Stirbet and Govindjee 2012). Radish growing under potassium deficiency showed a clear I-step, which indicate that PQH_2 oxidation might be slowed down, yet not entirely blocked. This may result from K^+ and Mg^{2+} role in balancing the H^+ efflux to thylakoid lumen. It was suggested that Cl^- efflux from and Mg^{2+} and K^+ influx to the stroma are the major counterion fluxes balancing the light-driven H^+ efflux to thylakoid lumen (Pottosin and Shabala 2016, Lyu and Lazár 2017). At physiological ionic conditions (at the concentration of $K^+ > 5$ mM), K^+ influx appears to be more important for the charge balance, while Mg^{2+} efflux most likely plays a regulatory role, connecting stromal metabolism to light reactions. At low K^+ concentration (< 5 mM), Mg^{2+} flux will dominate the charge balance (Hind *et al.* 1974, Pottosin and Shabala 2016). The ability to balance lumen pH in low K^+ availability could have been the reason for the employment of the downregulation feedback which leads to the rearrangement of PSII complexes and an increase in the thermal dissipation of absorbed energy rather than limiting PQH_2 oxidation at the Cyt b_6f complex in K-deficient radish.

Overall, our results showed that two hybrid cultivars of radish reacted to potassium deficiency in a different manner. The Fluo cultivar showed a greater tolerance to this type of stress as compared to the Suntella cultivar, by maintaining the CO_2 assimilation at the level of the control sample throughout the stress period. Our results did not support a negative effect of K deficiency on the transpiration rate and did not show stomatal limitation as the main reason for the reduction of net photosynthetic rate in this type of stress in radish. We conclude that a moderate K-deficiency stress impairs the plant's ability to regulate stomatal closure. Ideally, these findings should be replicated in a study where plants are subjected to various levels of potassium deficiency.

Results of our research demonstrate the effect of potassium deficiency on the photochemical phase of photosynthesis in both tolerant and sensitive cultivars of radish. The analyses lead to the suspicion that low K^+ availability impaired the balance of the H^+ influx to thylakoid lumen and increased its acidification, which triggered the downregulation mechanism leading to the rearrangement of PSII complexes by LHCII detachment and an increase in energy dissipation, yet without preventing the PQH_2 oxidation at the Cyt b_6f complex. However, the mechanisms of K^+ deficiency underlying processes reflected in Chl fluorescence induction curve and the JIP-test analysis remain speculative. Future investigations are necessary to validate the conclusions that can be drawn from this study. Despite the limitations of these findings, they add to a growing corpus of research showing the impact of nutrient deficiency stress on the efficiency and structure of the photosynthetic apparatus.

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Appendix. Definitions and equations of JIP-test parameters according to Strasser *et al.* (2004), Ref. [1]; Yusuf *et al.* (2010), Ref. [2]; and Stirbet and Govindjee (2011), Ref. [3].

Parameter	Other name(s)	Formula	Description	Ref.
F_o	F_0, F_{open}	$F_{0.05ms}$	First fluorescence value measured after the onset of actinic illumination and used as initial value for further calculations	[1], [3]
F_k		$F_{0.3ms}$	Fluorescence value at t = 0.3 ms	[2]
F_j		F_{2ms}	Fluorescence value at t = 2 ms	[1]
F_i		F_{30ms}	Fluorescence value at t = 30 ms	[1]
F_m	F_p, F_{max}	$F_{\approx300ms}$	Maximal fluorescence value recorded under saturating illumination; the peak of OJIP curve	[1], [3]
T_{FM}	t_{Fmax}, t for F_m		Time (in ms) to reach maximal fluorescence F_m	[1]
A_m	Area		Area between OJIP curve and line $F = F_m$	[1]
F_v		$F_v = F_m - F_o$	Maximal variable fluorescence	[1]
V_j		$V_j = (F_j - F_o)/(F_m - F_o)$	Relative variable fluorescence at t = 2 ms	[1]
V_i		$V_i = (F_i - F_o)/(F_m - F_o)$	Relative variable fluorescence at t = 30 ms	[1]
M_0		$M_0 = 4(F_k - F_o)/(F_m - F_o)$	Approximate initial slope of the fluorescence transient normalized on the maximal variable fluorescence F_v	[1], [2]
S_m		$S_m = A_m/(F_m - F_o)$	Normalized area to F_m	[1]
N		$N = S_m \times M_0 \times (1/V_j)$	Turnover number; number of Q_A reduction events between time 0 and T_{FM}	[1]
ϕ_{Po}	F_v/F_m, TR_0/ABS	$\phi_{Po} = F_v/F_m = 1 - (F_o/F_m)$	Maximum quantum yield of primary PSII photochemistry	[1], [3]
ϕ_{Eo}	ET_0/ABS, ϕET_0	$\phi_{Eo} = 1 - F_j/F_m = \phi_{Po}(1 - V_j)$	Quantum yield of electron transport	[1], [3]
ϕ_{Ro}	RE_0/ABS	$\phi_{Ro} = 1 - F_i/F_m = $ $= \phi_{Po} \times (1 - V_i)$	Quantum yield of electron transport flux until PSI electron acceptors	[1], [3]
ψ_{Eo}	ET_0/TR_0, ψET_0	$\psi_{Eo} = 1 - V_j$	Efficiency/probability with which an electron trapped in PSII RC is transferred beyond Q_A^-	[1]
δ_{Ro}	RE_0/ET_0, δRE_0	$\delta_{Ro} = (1 - V_i)/(1 - V_j)$	Efficiency/probability with which an electron from Q_B is transferred until PSI acceptors	[3]
RC/ABS	$\gamma RC/(1 - \gamma RC)$	$RC/ABS = \phi_{Po} \times V_j/M_0$	Number of Q_A reducing RCs per PSII antenna chlorophyll	[2], [3]
TR_0/RC		$TR_0/RC = M_0 \times (1/V_j)$	Trapped energy flux per RC at t = 0	[1]
ET_0/RC		$ET_0/RC = M_0 \times (1/V_j) \times \psi_{Eo}$	Electron transport flux further than Q_A^- per RC	[1], [3]
RE_0/RC		$RE_0/RC = (M_0/V_j) \times (1 - V_i)$	Electron transport flux until PSI acceptors per PSII RC	[3]
PI_{abs}		$PI_{abs} = (RC/ABS) \times$ $\times (\phi_{Po}/1 - \phi_{Po}) \times (\psi_{Eo}/1 - \psi_{Eo})$	Performance index (potential) for energy conservation from photons absorbed by PSII antenna to the reduction of Q_B	[1], [2], [3]
PI_{tot}	PI total	$PI_{tot} = PI_{abs} \times \phi_{Ro}/(1 - \phi_{Ro})$	Performance index (potential) for energy conservation from photons absorbed by PSII antenna until the reduction of PSI acceptors	[2], [3]
ABS/CS_0		$\approx F_o$	Absorption flux per CS, approximated by F_o	[1]
RC/CS_0		$RC/CS_0 = \phi_{Po} \times (V_j/M_0) \times$ $\times (ABS/CS_0)$	Density of active PSII RCs per cross-section (CS)	[1], [3]