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Physiological and molecular responses of maize (*Zea mays* L.) plants to drought and rehydration

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Abstract

Physiological and molecular responses of maize seedlings (*Zea mays* L. cv. Troinaya sladost) to 5-d drought and rehydration for 48 h were investigated. Plant water status was determined by a new method of water potential measurement in mesophyll cells' apoplast in substomatal cavity (ψ_{wa}). Drought caused the changes in water status, plant growth, the rates of photosynthetic CO_2/H_2O gas exchange, and metabolism of carbohydrates and proline. The increase in carbohydrate and proline content under drought was observed simultaneously with the decline in ψ_{wa}. Rewatering of seedlings for 24 and 48 h resulted in restoration of growth, rapid increase in ψ_{wa} as well as in the rates of photosynthetic gas exchange, and a sharp decline in the content of soluble sugars and proline. Data on close correspondence between the changes in osmolyte content and ψ_{wa} under drought and recovery support the assumption that osmolytes might participate in regulation of ψ_{wa}.

Additional key words: drought tolerance; drought stress; malondialdehyde; photosynthesis; pigments.

Introduction

Drought is one of the most important abiotic stressors affecting plant growth, development, and productivity. In the future, global warming and the growth of human population can lead to reduction of water resources and an increase in arid and semiarid areas. Therefore, the study of mechanisms of plant adaptation and tolerance to drought, as well as of the ability to recover after water deficit is an important task for modern research.

Physiological and developmental plant responses to drought were shown to occur by reprogramming gene expression and metabolism (Reddy *et al*. 2004, Chaves *et al*. 2009, Hayano-Kanashiro *et al.* 2009, Zhang *et al.* 2014). Responses to drought stress depend on plant species, the stage of development, the rate of dehydration, and the duration and severity of stress (Reddy *et al*. 2004, Chaves *et al*. 2009). To elucidate plant ability to survive under drought, it is of importance to study the physiological, biochemical, and genetic basis of adaptation and tolerance as well as the mechanisms of recovery under rehydration. Plant tolerance to water deficit requires the ability to maintain functions under unfavorable water conditions and to recover water status and functions rapidly after rewatering. Recent studies showed that recovery phase is as important as the stress treatment since the efficient recovery affects further plant growth and development (Chen *et al*. 2016, Kosová *et al.* 2018). Significant variations in responses to drought stress and in mechanisms of recovery after rehydration were revealed in varieties with different drought tolerance (Hayano-Kanashiro *et al.* 2009, Cruz de Carvalho *et al*. 2011, Foster *et al*. 2015, Sun *et al*. 2016, Kosová *et al.* 2018). Tolerant maize genotypes were shown to recover more efficiently after drought as compared to sensitive ones. Some less tolerant maize cultivars were unable to activate their acclimation mechanism and to restore after drought (Cruz de Carvalho *et al*. 2011). These data revealed broad plasticity of maize in response to water stress and showed that in crop plants, capacity to recover from previous water deficit should be clarified further.

The changes in carbohydrate metabolism under drought conditions are closely related to photosynthesis and transpiration and are of great importance for stabilization of water balance of plants (Hare *et al*. 1998, Tarchevsky 2001, Reddy *et al.* 2004, Chaves *et al*. 2009). Previously, we observed a sharp increase in the content of reducing sugars and proline simultaneously with significant reduction in the rate of photosynthesis and transpiration during the adaptation of maize seedlings to drought (Nikolaeva *et al.* 2017). Accumulation of osmolytes in the cells is known to lead to the formation of concentration gradient between the inside and outside cell compartments. This concentration gradient might create favorable conditions for the transfer of osmolytes from the photosynthesizing cells into apoplast. Recently, a new and noninvasive method of direct measurement of water potential of mesophyll cells' apoplast in substomatal cavity (ψ_{wa}) was described (Voronin *et al*. 2017). In addition, the new method permits

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Abbreviations: Car – carotenoids; Chl – chlorophyll; DM – dry mass; DSP – drought-stressed plants; *E* – transpiration rate; FC – field capacity; FM – fresh mass; LPO – lipid peroxidation; MDA – malondialdehyde; P_N – net photosynthetic rate; R_D – respiration rate; RH – relative humidity; ROS – reactive oxygen species; RWC – relative water content; ψ_{wa} – water potential of mesophyll cells' apoplast in substomatal cavity.

measurements on an intact leaf in parallel to measurements of net photosynthetic rate (P_N), transpiration (E), and respiration rate (R_D). Determination of ψ_{wa} in the needles of water-stressed pine showed that drought caused the reduction of its value (Voronin *et al.* 2018). All these data suggest that an increase in the concentration of osmotically active agents might lead to a decrease in $\psi_{wa.}$ The aim of this study was to qualify a hypothesis that the accumulation of soluble sugars and proline under drought probably leads to a decrease in ψ_{wa} and, on the contrary, the reduction of osmolyte content after rewatering results in an increase in ψ_{wa} alongside with restoration of the leaf water status. To this end, we studied the effect of drought and subsequent rewatering on the water status [relative water content (RWC) and ψ_{wa}] and growth, activity of CO_2/H_2O gas exchange, pigment content, as well as metabolism of carbohydrates and proline in the leaves of maize plants at the seedling stage.

Materials and methods

Plant material and growth conditions: Experiments were conducted with maize plants (*Z. mays* L. cv. Troinaya sladost). The seeds were purchased from the *Russkii ogorod* company. The maize cultivar used in the experiments is known to be high-yielding and tolerant to drought. Its grains contain high amount of sugars, proteins, and vitamins. The seeds were treated with 15% hydrogen peroxide for 30 min and then rinsed with distilled water. The seeds were germinated for 3 d at 25°C. The seedlings were grown in a mixture of sand and sod-podzolic soil at a mass ratio of 2:1 in 5-L pots (5 kg of soil per pot). After mixing of the two soils, the texture was 76% sand, 20% silt, and 4% clay. Bulk density of the soil was 1.30 g cm^{-3}. Field capacity (FC) of the soil was 33.4%. Five seeds were sown in each pot. After 4 d, the plants were thinned to three per pot. Plant watering up to 60% of FC was done manually after pot weighting. The irradiance at the top of plants was of 200 μmol(photon) m^{-2} s^{-1} PAR during 16-h photoperiod, the temperature was maintained at 25/20°C (day/night). On the 8th day after shoot emergence, the plants were divided into two groups – control (60 plants) and treated (60 plants), and watering of treated plants was stopped. Control plants were watered daily to maintain soil water content at 60% of FC. Five days of progressive drought reduced water content to 26.3% FC. After 5-d drought, the sampling of control and drought-stressed plants (DSP) was done simultaneously. Subsequently, DSP were watered to restore FC up to 60%. To evaluate a recovery response, the sampling was done after 24 and 48 h of rewatering. Samples were taken from the middle part of the third leaf that completed growth two days after the beginning of drought. Sampling was made on 15 seedlings. All samples for biochemical analysis were immediately frozen in liquid nitrogen and stored at –80°C. In the Tables 1–4 and Fig. 1, 5-d drought and rewatering for 24 and 48 h were referred to as I, II, and III treatments, respectively.

Relative water content (RWC): To calculate RWC, we determined fresh mass (FM) of leaf sample, turgid mass (TM) after full saturation for 24 h, and mass of samples after drying at 80°C for 2 d (DM). RWC was determined as: RWC [%] = 100 × (FM – DM)/(TM – DM).

Water potential of mesophyll cells' apoplast in substomatal cavity (ψ_{wa}) was determined in the attached leaf by means of a new method using the instruments to assess photosynthetic CO_2/H_2O gas exchange [a single-channel infrared gas analyzer *LI-820* (*LI-COR*, *Inc*., Lincoln, NE, USA] (Voronin *et al*. 2017). The method is based on the determination of relative humidity (RH) above the leaf surface that reduces E to zero. This value is equal to RH in the substomatal cavity. Determination of RH values makes it possible to calculate ψ_{wa} at the interface between aqueous and gaseous phases of mesophyll cells' apoplast in substomatal cavity. RH at the air stream entering the leaf chamber was maintained by dew point generator *LI-610* (*LI-COR*, *Inc*., Lincoln, NE, USA) and determined using a psychrometric sensor *HMP50* (*Vaisala INTERCAP*, Finland). Equilibrium pressure of vapor over the surface of an aqueous solution is related to the chemical potential of water by the following equation: $e = e_o \times \exp[\psi_w \times V/(R \times T)]$, where e is equilibrium pressure of vapor over aqueous solution, e_o is pressure of saturated vapor over the surface of pure water ($\psi_w = 0$) at absolute temperature T, R is absolute gas constant of 8.31441 J mol^{-1} K^{-1}, T is absolute temperature (K), and V is molar volume of water (18 cm^3 mol^{-1}). Therefore, $\psi_w = (R \times T/V) \times \ln(e/e_o)$. Water potential is expressed in J m^{-3} or Pa.

By definition, relative humidity is described with the formula: RH [%] = $(e/e_o) \times 100$, where e_o is pressure of saturated vapor [Pa] and e is real pressure of vapor [Pa] at temperature t [°C]. The value of ψ_{wa} was expressed in MPa. It takes <1 h to measure leaf ψ_{wa}.

FM of plant aboveground part: The plant growth was characterized by measurements of FM of plant aboveground part of 15 seedlings. FM of the aboveground part was calculated per plant.

CO_2/H_2O gas exchange: P_N was measured in the third attached leaf in a clamp-on leaf chamber having useful volume of 38 × 14 × 8 mm^3 with a single-channel infrared gas analyzer *LI-820* (*LI-COR*, *Inc*., Lincoln, NE, USA) in an open system with 380 ppm CO_2 concentration at irradiance of 1,200 μmol(photon) m^{-2} s^{-1} (Voronin 2014). E was calculated using the difference of RH at the inlet and outlet of the leaf chamber. R_D of leaf was measured after switching the light off. CO_2/H_2O gas-exchange measurements were calculated per leaf area. Leaf gas exchange was measured from 8 to 14 h.

Pigment extraction and quantification: Chlorophylls (Chl) and carotenoids (Car) were extracted in 80% acetone. After centrifugation at 1,000 × *g* for 15 min, absorbance of extracts was read at 663, 646, and 470 nm using a *Genesys 10 UV Scanning* spectrophotometer (*Thermo Scientific*, USA). Pigment concentration was calculated according to Lichtenthaler (1987) and expressed as mg g^{-1}(FM).

Soluble sugar content: Leaf sample (0.20–0.30 g of FM) was fixed in boiling 96% ethanol. Soluble carbohydrates were extracted three times with hot 80% ethanol (50–60°C). After centrifugation at 1,000 × *g* for 15 min, the supernatant was evaporated to dryness. The residue was dissolved in 3 cm^3 of warm water and 3 cm^3 of chloroform was added to remove pigments. After centrifugation, the water phase was separated and purified using 0.15 M $Ba(OH)_2$ and 10% (w/v) $ZnSO_4$. The amount of fructose and sucrose in the purified extracts was determined by resorcinol method (Turkina and Sokolova 1971). For glucose determination, a standard enzyme system (glucose oxidase-peroxidase, *Sigma-Aldrich*, St. Louis, MO, USA) was used. Content of sucrose was expressed as mg g^{-1}(FM). Content of glucose and fructose was expressed as μg g^{-1}(FM).

Starch content was determined in residue after the removal of soluble sugars according to modified method of Dubois (Pisarenko 1971). Starch was extracted with 52% perchloric acid, its content was determined as glucose equivalents at 490 nm using a *Genesys 10 UV Scanning* spectrophotometer (*Thermo Scientific*, USA). We used D-glucose solution, 1 mg cm^{-3}, as a standard (*Sigma-Aldrich*, St. Louis, MO, USA). Starch content was expressed as mg g^{-1}(FM).

Proline content: Free proline was extracted twice from the plant sample (0.3 g of FM) with 3% (w/v) 5-sulfosalicylic acid. Homogenate was centrifuged at 1,000 × *g* for 15 min. Proline content in the supernatant was determined using the method described in Bates *et al*. (1973). The supernatant (1 cm^3) was treated with 1 cm^3 of glacial acetic acid and 1 cm^3 of ninhydrin reagent (1.25 g ninhydrin dissolved in 30 cm^3 glacial acetic acid and 20 cm^3 of 6 M phosphoric acid). The reaction was carried out for 1 h during the incubation of samples in a boiling water bath. Next, the samples were rapidly cooled on ice, mixed with 3 cm^3 toluene, and vigorously shaken. Absorbance of pink-red toluene fraction was measured at 520 nm using a *Genesys 10 UV Scanning* spectrophotometer (*Thermo Scientific*, USA). Proline concentration was determined using a calibration curve and expressed as μmol g^{-1}(FM).

Lipid peroxidation (LPO) level was assessed by means of test, based on the interaction of thiobarbituric acid with MDA, the most abundant end product of LPO. MDA content was determined according to Heath and Packer (1968). Leaf sample (0.20–0.50 g of FM) was ground in a mortar in 2 cm^3 of 0.1% trichloroacetic acid. Homogenate was centrifuged at 1,000 × *g* for 10 min, and 1 cm^3 of supernatant was mixed with 4 cm^3 of solution containing 0.5% (w/v) thiobarbituric acid and 20% (w/v) trichloroacetic acid. The mixture was heated in a boiling water bath for 30 min and then rapidly cooled on ice and centrifuged at 1000 × g for 15 min. Next, absorbance of samples was measured at 532 and 600 nm. MDA concentration was calculated after subtraction of nonspecific absorbance at 600 nm using extinction coefficient of 155 mM^{-1} cm^{-1}. MDA content was expressed as nmol g^{-1}(FM).

Statistical analysis: Three independent experiments were performed. All measurements were performed three times for each treatment. The means and standard errors (SE) were calculated using *SigmaPlot 12.0* statistical program (*Systat Software Inc*.). Comparisons of parameters were made between treatments using analysis of variance (*ANOVA)* with a post hoc *Tukey*'s test for pairwise comparison. Differences were considered significant at $P<0.05$.

Results

RWC and water potential of mesophyll cells' apoplast in substomatal cavity: Five days of gradual dehydration led to the changes in water status of plants. In treated seedlings, RWC decreased by 11.5% as compared with control (Table 1). During the experiments, RWC value in the leaves of control plants did not change significantly. Under drought, ψ_{wa} of mesophyll cells' apoplast in substomatal cavity in the leaves of DSP decreased nearly 2-fold (Table 1). On rewatering, plants recovered fully in terms of RWC and ψ_{wa}.

FM of plant aboveground part: Under drought, FM of the aboveground part of maize seedlings calculated per plant decreased by 65% as compared with control (Table 1). After rewatering for 24 and 48 h, the difference between the control and treated seedlings reduced to 30 and 15%, respectively.

CO_2/H_2O gas exchange: After 5-d drought, the rates of P_N and E in the leaves of DSP decreased almost the same as compared with control (Fig. 1*A*,*B*) and increased rapidly to the control value in response to rehydration (24 h). Under the influence of drought, the rate of R_D increased 2-fold as compared with the control values (Fig. 1*C*). After 24 h from the onset of rehydration, the rate of R_D was nearly identical to that in the control leaves and, after 48 h, it did not differ from the control level.

Table 1. Changes in water potential of mesophyll cells' apoplast in substomatal cavity (Ψ_{wa}), relative water content (RWC), and fresh mass (FM) in maize leaves after 5-d drought stress (DS) (I) and rewatering for 24 (II) and 48 h (III). The values are means ± SE of four replicates. *Different letters* indicate significant differences between control and treatments at $P<0.05$.

Treatment	Ψ_{wa} [MPa]		RWC [%]		FM [g]	
	Control	DS	Control	DS	Control	DS
I	–48.0 ± 3.0^a	–91.0 ± 4.0^b	98.2 ± 1.8^a	86.9 ± 0.9^b	2.15 ± 0.08^a	0.76 ± 0.05^b
II	–45.0 ± 3.0^a	–45.0 ± 3.0^a	97.9 ± 2.1^a	96.8 ± 1.6^a	2.50 ± 0.10^a	1.76 ± 0.08^b
III	–40.0 ± 1.0^a	–40.0 ± 2.0^a	98.7 ± 1.4^a	97.9 ± 2.0^a	2.40 ± 0.12^a	2.05 ± 0.10^b

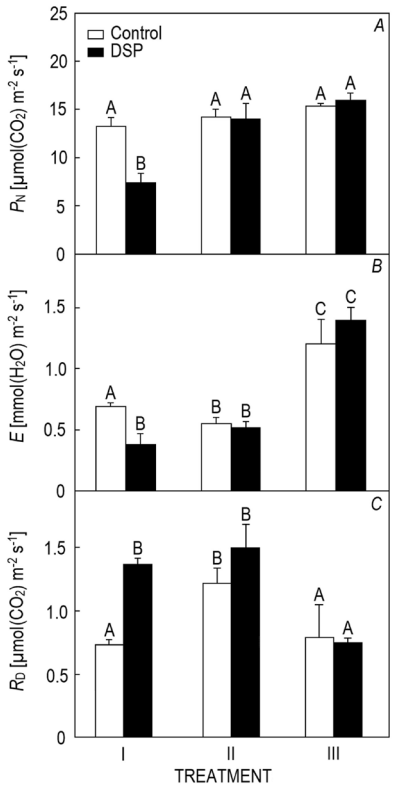

Fig. 1. Effect of 5-d drought stress (DS) (I) and rewatering for 24 (II) and 48 h (III) on net photosynthetic rate (P_N) (*A*), transpiration rate (*E*) (*B*), and dark respiration rate (R_D) (*C*) in maize leaves. Bars indicate standard errors, $n = 4$. Columns with *different letters* are significantly different at $P<0.05$. Control – control plants, DSP – drought-stressed plants.

Pigment content: Under influence of drought, Chl (*a*+*b*) content increased by 19%, and the content of Car did not change (Table 2). As a result of recovery, the Chl content in the leaves of treated plants declined to the control level.

Soluble sugar content: Five days of drought had a significant effect on carbohydrate metabolism. The content of glucose and fructose increased 9 and 4.2 times, respectively, as compared with control plants (Table 3). In the leaves of DSP, sucrose content rose 2.5 times. Rewatering for 24 h resulted in a significant decrease in the content of reducing sugars (glucose and fructose) and sucrose. After 48 h of recovery, glucose and fructose content exceeded the control level by 23 and 38%, respectively. After 24 h from the onset of rewatering, the sucrose content in the leaves of DSP did not differ from that in control.

Starch content: In DSP, the starch content decreased insignificantly (Table 3). After rewatering (24 h), it was close to that in control and then decreased by 19% (48 h).

Proline content: In the leaves of DSP, the proline content increased 13-fold as compared with control in parallel with the changes in the content of soluble sugars (Table 4). After the onset of rewatering (24 h), a 10-fold decrease in the proline content was observed. After 48 h, it was similar in DSP and control plants.

MDA content: Under drought, MDA content exceeded the control value by 30% (Table 4). After 24 h of recovery, MDA content in DSP decreased, however, it remained 19% higher than that in the leaves of control plants. After 48 h, it reached the control level.

Discussion

Maize is considered to be highly sensitive to water deficit (Ghannoum 2009, Benešová *et al*. 2012). For this reason, high maize yield may be obtained only under sufficient water supply. In our experiments, RWC, a widely used indicator of plant sensitivity to dehydration, decreased more than 10% (Table 1). Such water deficit might be considered moderate, according to the classification of Hsiao (Hsiao 1973). In the treated plants, leaf rolling was observed. These changes are adaptive morphological traits restricting transpiration and promoting water retention in leaf tissues (Srivalli *et al.* 2003). Under progressive drought, ψ_{wa} decreased significantly alongside with considerable changes in CO_2/H_2O gas exchange (Table 1, Fig. 1). After rehydration, values of RWC and ψ_{wa} increased rapidly testifying to the restoration of plant water status. A rapid increase in leaf water potential was observed in three maize genotypes ten hours after the recovery irrigation (Hayano-Kanashiro *et al.* 2009).

Drought also caused a significant decrease in FM of the aboveground part of the treated plants. It resulted from the inhibition of young leaf growth due to reduction in cell division and enlargement (Avramova *et al.* 2015). After rewatering, DSP resumed their growth. However, 48 h after the onset of rewatering, FM of the aboveground part calculated per plant still did not attain the control level. The inhibition of growth, an important physiological characteristic, is known to be one of the earliest responses to water deficit (Maksimov 1939, Avramova *et al.* 2015).

Table 2. Contents of chlorophyll (Chl) (*a*+*b*) and carotenoids (Car) in maize leaves after 5 d-drought stress (DS) (I) and rewatering for 24 (II) and 48 (III) h. The values are means ± SE of four replicates. *Different letters* indicate significant differences between control and treatments at $P<0.05$.

Treatment	Chl (*a*+*b*) [mg g^{-1}(FM)]		Car [mg g^{-1}(FM)]	
	Control	DS	Control	DS
I	2.93 ± 0.03^a	3.49 ± 0.06^b	0.44 ± 0.02^a	0.48 ± 0.02^a
II	3.02 ± 0.05^a	3.36 ± 0.04^b	0.46 ± 0.01^a	0.50 ± 0.03^a
III	3.40 ± 0.04^a	3.33 ± 0.03^a	0.54 ± 0.02^a	0.54 ± 0.02^a

Table 3. Contents of sucrose, glucose, fructose, and starch in maize leaves after 5 d-drought stress (DS) (I) and rewatering for 24 (II) and 48 (III) h. The values are means ± SE of five replicates. *Different letters* indicate significant differences between control and treatments at $P<0.05$.

Treatment	Sucrose [mg g^{-1}(FM)]		Glucose [μg g^{-1}(FM)]		Fructose [μg g^{-1}(FM)]		Starch [mg g^{-1}(FM)]	
	Control	DS	Control	DS	Control	DS	Control	DS
I	2.18 ± 0.17^a	5.48 ± 0.38^b	59.3 ± 2.9^a	533.0 ± 25.0^b	156.0 ± 7.0^a	656.4 ± 31.4^b	5.30 ± 0.42^a	4.15 ± 0.33^b
II	2.10 ± 0.14^a	2.40 ± 0.16^a	48.4 ± 2.0^a	117.0 ± 5.0^b	170.0 ± 8.2^a	285.6 ± 12.9^b	6.22 ± 0.49^a	6.75 ± 0.54^a
III	2.38 ± 0.16^a	2.45 ± 0.17^a	53.6 ± 2.4^a	66.1 ± 3.0^b	165.0 ± 7.7^a	228.0 ± 10.1^b	7.30 ± 0.42^a	6.13 ± 0.42^b

Table 4. Contents of proline and malondialdehyde (MDA) in maize leaves after 5 d-drought stress (DS) (I) and rewatering for 24 (II) and 48 h (III). The values are means ± SE of four replicates. *Different letters* indicate significant differences between control and treatments at $P<0.05$.

Treatment	Proline [μmol g^{-1}(FM)]		MDA [nmol g^{-1}(FM)]	
	Control	DS	Control	DS
I	0.14 ± 0.01^a	1.82 ± 0.12^b	28.2 ± 1.2^a	36.8 ± 1.6^b
II	0.13 ± 0.01^a	0.19 ± 0.02^b	25.0 ± 1.2^a	29.7 ± 1.0^b
III	0.13 ± 0.01^a	0.14 ± 0.01^a	27.3 ± 1.6^a	28.3 ± 1.0^a

Previously, it was demonstrated that elongation of young maize leaves was extremely sensitive to changes in the plant water status. Virtually instant growth acceleration of the young leaves was observed after raising the water potential of the root medium (Acevedo *et al*. 1971). At the same time, it was shown that the growth rate fully recovered only when water stress was not severe.

Along with the inhibition of growth, the suppression of photosynthesis is also a typical response to drought (Pustovoitova and Zholkevich 1992, Tarchevsky 2001, Chaves *et al*. 2009). In our experiment, water stress resulted in a nearly similar decrease in P_N and *E*. These data show that the inhibition of photosynthesis was most likely caused by a stomatal factor. Rehydration for 24 h resulted in a rapid recovery of photosynthetic rate and transpiration (Fig. 1*A*,*B*). Our data are consistent with previous studies concerning the recovery of photosynthetic capacity upon rewatering. Total recovery of P_N in the leaves of DSP was observed within 24–48 h after rewatering (Pelleschi *et al*. 1997, Foyer *et al*. 1998). Rewatering for 12 h of three Mexican maize landraces subjected to a progressive water deficit for 17 d resulted in the recovery of P_N to the level that exceeded that in the control plants (Hayano-Kanashiro *et al.* 2009).

Under drought, the rate of R_D increased markedly. This suggests that the role of R_D increased as water stress developed. A complete restoration of R_D was achieved after 24 h of rewatering (Fig. 1*C*). Previously, different effects of water stress on R_D (from decrease to stimulation) were noted (Flexas *et al*. 2006).

The response of plant pigments to drought is known to depend on the severity of stress, the stage of leaf development, and leaf susceptibility. Under the influence of drought, the content of pigments in maize leaves may increase (Avramova *et al*. 2015) as well as decrease (Chen *et al.* 2016). We observed an increase in the content of Chl (19%) in the leaves of DSP (Table 2). The changes in the content of Chl may be caused by the elevation of the level of transcripts encoding enzymes involved in the tetrapyrrole synthesis (Avramova *et al*. 2015). The retention of pigment content under drought is an indicator that photosynthetic membranes remained unaffected.

A decrease in photosynthetic activity in maize leaves proceeded simultaneously with the accumulation of reducing sugars, sucrose, and proline (Fig. 1*A*, Tables 3, 4). It is worth noting that the increase in glucose content exceeded significantly that of fructose and sucrose. The accumulation of hexose is one of the earliest metabolite changes in maize leaves under water deficit (Foyer *at al*. 1998, Kim *et al.* 2000, Sicher and Barnaby 2012). The increase in the concentration of soluble sugars is also a typical physiological response that is of importance for osmotic adjustment (Morgan 1984). Glucose and fructose are the sources of carbon and energy for the plant cells as well as the important signaling molecules; they may also play a key role in the integration of cellular responses at the level of the whole plant (Couée *et al.* 2006).

The accumulation of reducing sugars (glucose and fructose) was shown to be caused by an increase in the activity of soluble acid invertase (Kim *et al.* 2000). Moreover, photosynthetic capacity of maize leaves is likely to account for hexose accumulation observed under water stress (Foyer *et al.* 1998). The increase in the content of soluble sugars in the leaves may also, to a certain extent, result from hydrolysis of plastid starch (Hare *et al*. 1998, Lawlor and Cornic 2002, Muhammadkhani and Heidari 2008). The main cause of sucrose accumulation was the growth inhibition observed under drought.

Proline is known to accumulate in plants under drought and other stresses (Hare *et al.* 1998, Kuznetsov and Shevyakova, 1999, Kaur and Asthir 2015). Due to osmotic, osmoprotective, and antioxidant properties of proline, its accumulation is of great importance for improving plant resistance. Furthermore, the proline biosynthesis is accompanied by consumption of NADPH, thus diminishing the overreduction of electron transport

chain of photosynthesis under drought (Hare *et al.* 1998, Sharma *et al.* 2011, Kaur and Asthir 2015). The unique role of proline in maintaining plant growth at low water potential was shown (Sharma *et al*. 2011). Soluble sugars and proline ensure the cell osmotic balance and stabilize cell membranes. Moreover, compatible osmolytes play an essential role in neutralizing reactive oxygen species (ROS) (Hare *et al.* 1998, Kuznetsov and Shevyakova 1999, Reddy *et al.* 2004, Kaur and Asthir 2015). In our experiments, the increase in carbohydrate and proline content under drought in maize seedlings was observed simultaneously with the decline in ψ_{wa} value (Tables 1, 3, 4). Accumulation of soluble sugars and proline is likely to increase concentration gradient between the inside cell compartment and apoplast, osmolyte transport into apoplast, thus decreasing ψ_{wa} value.

After rewatering, an increase in ψ_{wa} in the leaves of treated plants was accompanied by a sharp decline in carbohydrate and proline content (Tables 3, 4). A substantial decrease in the content of soluble sugars suggests their active usage as carbon skeletons for nitrogen assimilation, growth processes, respiration, and intensive transport to the growing leaves. Under recovery, proline could be rapidly utilized as a source of carbon, nitrogen, and reducing power (Hare *et al.* 1998). The early recovery from water stress was shown to be a critical period for drought tolerance as oxidative stress might be activated (Mittler and Zilinskas 1994, Sgherri *et al.* 2000, Hayano-Kanashiro *et al*. 2009). The accumulation of proline and reducing sugars could promote plant repair ability by activation of ROS scavenging system. Probably, in our experiments, the higher concentration of reducing sugars in the leaves of treated plants after rewatering for 48 h might minimize the damaging effect of ROS on enzyme activity and cell structure (Hayano-Kanashiro *et al*. 2009, Sun *et al*. 2016).

In the leaves of DSP, despite the significant increase in soluble carbohydrates and proline content, the MDA content rose. It is likely that the process of LPO was not severe enough to inhibit pigment synthesis and damage photosynthetic membranes. Under recovery, MDA content declined simultaneously with the restoration of plant water status and normalization of the photosynthetic gas exchange (Table 4, Fig. 1*A*,*B*).

In conclusion, 5-d drought caused a series of changes in water status (RWC and ψ_{wa}), plant growth, rates of photosynthetic CO_2/H_2O gas exchange, and R_D, as well as in metabolism of carbohydrates and proline. Subsequent rewatering of maize seedlings for 24 and 48 h resulted in restoration of seedling growth as well as in a rapid increase in ψ_{wa} and the rate of photosynthetic gas exchange. Simultaneously, a sharp decline in the content of soluble sugars and proline was observed. After 48 h of rewatering, the MDA content decreased to the control level. A close correspondence between the changes in the content of osmolytes (glucose, fructose, and proline) and ψ_{wa} under drought and recovery after rewatering was revealed. Thus, the data obtained support our assumption that the accumulation of soluble sugars and proline in the leaves under drought might lead to a decrease in ψ_{wa} while the reduction in the osmolyte content after rewatering results in an increase in ψ_{wa}. Further studies are needed to clarify the mechanisms regulating ψ_{wa} under drought and recovery.

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