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Differences between C3 (*Hordeum vulgare* L.) and C4 (*Panicum miliaceum* L.) plants with respect to their resistance to water deficit

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Abstract

C4 photosynthetic pathway has an advantage over the C3 photosynthesis in arid environments due to a CO₂ concentrating mechanism, enabling prolonged stomatal closure. However, the question arises if the metabolic limitations do not outweigh the stomatal limitations in the case of a severe water deficit. The aim of this research is to investigate the differences of drought resistance between C3 plant spring barley (*Hordeum vulgare* L.) and C4 plant common millet (*Panicum miliaceum* L.) according to the impact of relatively slight and severe drought on growth, photosynthetic apparatus and membrane oxidative damage. The growth of the millet plants was impaired more seriously than that of the barley plants. The height of slight or severe drought-treated barley decreased by 5.0% and 15.4% of control level, compared to the height decreased by 18.7% and 28.9% respectively for the millet plants. No statistically significant decrease in shoot dry weigh was detected after slight drought, whereas the strong drought reduced accumulation of shoot dry biomass of barley and millet by 12.7% and 17.7%, respectively. The photosynthetic rate of the millet plants was less affected in comparison to barley. Considerably lower stomatal conductance, transpiration rate, intercellular CO₂ concentration, lipid peroxidation and higher water use efficiency (WUE) were detected for the millet plants.

However, the growth and the photosynthetic rate of barley plants were more able to recover during the period of re-watering. Our results suggest that the advantages of the C4 photosynthetic pathway could lead to higher drought resistance due to lower oxidative stress (determined by an absence of photorespiration) and higher WUE. On the other hand, the contribution of non-stomatal and redox-independent limitations of growth remains to be elucidated.

Key words: *Hordeum vulgare*, *Panicum miliaceum*, drought, C4 pathway, oxidative stress.

Introduction

Water deficit is one of the worst disasters that affect human health and activities in the world. The changes in precipitation patterns and the expansion of water-logging or of drought affected areas are expected in the future due to global climate change (Xoconstle-Cazares et al., 2010). Breeding of drought-resistant plants can be an advantageous strategy to avoid drought-induced damage to agriculture. A suitable alternative to ordinary crops is C4 plants, such as maize, sorghum, millet, amaranth, etc. These plants are characterized by high productivity (Svirskis, 2009 a), nutritive value, and resistance to water deficit (Liu, Stutzel, 2004; Osborne, Freckleton, 2009). They dominate in arid and hot regions; approximately half of the world's grasses and only from 4 to 10% of all plant species use the C4 photosynthetic pathway. Nevertheless, high photosynthetic capacity and productivity of these plants determine their essential contribution to global primary production (Osborne, Freckleton, 2009).

The main reason why C4 plants have an advantage in arid environments lies in their photosynthetic pathway, which consists of several biochemical and structural adaptations (Ghannoum, 2009). Contrary to C3 plants that fix atmospheric CO₂ directly in mesophyll cells, CO₂ is firstly hydrated into bicarbonate and bound with phosphoenol pyruvate (PEP), producing dicarboxylic acid with four carbon atoms (oxaloacetate) in C4 plants. This type of carbon fixation is catalysed by enzyme phosphoenol pyruvate carboxylase (PEPC) and takes place in mesophyll cells that are situated around vascular tissue (Larcher, 1995). Oxaloacetate is converted into other carbon acids, that diffuse to the cells of bundle sheath, that are situated around a mesophyll layer. CO₂ is released, fixed by Rubisco and it then participates in the rest of the C3 carbon assimilation cycle. The division of CO₂ fixation into spatially distinct tissues serves as a CO₂ concentrating mechanism, due to these reasons: firstly, PEPC,

unlike Rubisco, has a much higher catalytic capacity and has no affinity to O₂; and, secondly, the thick walls of the bundle sheath cells secure a gaseous diffusion barrier. As a consequence, the bundle sheath is saturated with a sufficient amount of CO₂; photorespiration is very low in C4 plants, and photorespired CO₂ is returned to assimilation sites and refixed by Rubisco (Ghannoum, 2009).

The CO₂ concentrating mechanism, compared to the C3 photosynthetic pathway, ensures higher carbon assimilation rate and dry matter production under drought conditions, when stomata closure reduces CO₂ supply (Larcher, 1995). Consequently, higher stomatal resistance allows low transpiration rate and high WUE in C4 plants (Zhang, Kirkham, 1995). On the other hand, other factors begin to limit both C3 and C4 photosynthesis when drought stress persists. Stomatal resistance is the major CO₂ assimilation-limiting factor in the beginning of drought or during mild water stress (Cornic, Fresneau, 2002). However, metabolic limitations exceed the impact of stomata closure under severe drought (Lawlor, 2002; Flexas, Medrano, 2002; Ripley et al., 2007). It is suggested by Lawlor and Cornic (2002) that reduction in CO₂ fixation rate is mainly caused by limited ribulose-1,5-biphosphate (RuBP) regeneration, as a result of decreased ATP synthesis, when leaf relative growth rate is lower than 75%. Photorespiration is advantageous as an electron sink in this case: RuBP oxygenation maintains photosynthetic electron transport and ATP supply for carbon assimilation (Cornic, Fresneau, 2002).

Along with down-regulation of photosynthesis, oxidative stress is one of the major consequences of water shortage in plants (Dat et al., 2000; Xoconstle-Cazares et al., 2010). Accumulation of reactive oxygen species (ROS), such as superoxide, singlet oxygen, hydrogen peroxide and hydroxyl radical causes disruption of various biomolecules, and lipid peroxidation is considered to be the most intense process associated with drought oxidative damage (Zhang, Kirkham, 1995). Plant tissue dehydration induced ROS synthesis mainly originates from photosynthesis and is related to reduction of intercellular CO₂ concentration. Increase of photorespiration raises H₂O₂ production in peroxisomes. However, it protects PSII from overreduction and reduces superoxide synthesis during Mehler reaction in PSI, and singlet oxygen production from excited chlorophyll (Dat et al., 2000; Noctor et al., 2002).

The multiple role of photorespiration and the balance of stomatal vs. metabolic limitations of carbon assimilation in different photosynthetic pathways are not completely understood and raise the question if C4 plants are better adapted for severe drought (Lawlor, Cornic, 2002; Ripley et al., 2007). In practice, C4 plants, such as sorghum and amaranths proved to be more drought-resistant crops during rainless summer periods in recent years in Lithuania. The aim of this study is to evaluate the difference in C4 and C3 plant responses to water deficit. Drought resistance of the potential C4 crops, common millet (*Panicum miliaceum* L.), and the traditional crop spring barley (*Hordeum vulgare* L.) has been investigated and compared in this research. The impact of drought and the potential to recover after drought were examined by measuring plant growth, photosynthetic parameters

and intensity of membrane oxidative damage in shoots of plants affected by different levels of water shortage.

Materials and methods

Plant cultivation and drought treatment. A pot experiment was carried out in 2011 at Vytautas Magnus University, in a vegetation chamber with a controlled environment. Characteristics of the vegetation room were: photoperiod – 14 hours, average day/night temperature – 22°C/18°C, relative humidity – 65%, light intensity – 14000 Lx, provided by “Philips MASTER GreenPower CG T” 600 W lamps in combination with luminescence lamps. The Lithuanian cultivars of spring barley (*Hordeum vulgare* L.), ‘Aura DS’, and common millet (*Panicum miliaceum* L.), ‘Gelsvės’ were investigated. Sixteen seeds were sown to each vegetation pot (10 cm high, 12 cm in diameter and 8 drainage holes, 0.5 cm in diameter), filled with peat substrate (80 g of dry weight). After germination seedlings were thinned to 10 plants per pot.

Drought treatment was applied by inadequate watering, that lasted two weeks and was followed by a re-watering period of one week for the plants’ recovery. Drought treatment of the barley plants was started one week after emergence of the seedlings due to their faster ontogenesis compared to millet plants. Barley seedlings had 2–3 leaves unfolded (12–13 phenological stage according to the BBCH scale). The millet was drought-treated four weeks after germination, when 3 leaves were fully expanded (13 phenological stage according to the BBCH scale). Two levels of water shortage were used: 80% (slight drought) and 30% (severe drought) of the amount of water, used for the watering of the control plants. Soil humidity at the end of the drought treatment was: 60–70% for the control plants, 40–50% and 20–30% for relatively slight and severe drought, respectively. Soil humidity was evaluated by weighing soil samples immediately after taking from the pots and after drying them to a constant weight. The appropriate amount of water for the control plants was determined during the pre-experiments; watering with 70 ml of water twice a week was found to be optimal for both plant species. The watering was increased up to 80 ml three weeks after germination, because of higher requirements of the growing plants. Morphometric, photosynthetic and gas exchange parameters, as well as membrane oxidative damage were measured at the end of drought and recovery periods.

Morphometric measurements. Four plants per each pot were harvested to determine average shoot length and fresh biomass. After measuring the length, plants were immediately weighed to prevent tissue dehydration. Later, the same plants were dried in an electric oven at 70°C for 24 hours to determine the mean of dry biomass per plant.

Photosynthetic and gas exchange parameters. Photosynthetic rate, intercellular CO₂ concentration and transpiration rate were measured using a portable photosynthesis system LI-6400 (Li-Cor Inc., USA). Stomatal conductance and water use efficiency were calculated according to the manufacturer’s instructions, using the formulas:

$$G_s = 1/((1/TCond) - (0.5/BLCond)),$$
$$WUE = A/Tr,$$

where: G_s – stomatal conductance, when stomatal ratio from one side of the leaf to another is equal to 1, WUE – water use efficiency, $TCond$ – total conductance, $BLCond$ – boundary layer conductance, A – photosynthetic rate, and Tr – transpiration rate. $TCond$, $TLCond$, A and Tr are systemic variables of the apparatus, and are calculated automatically.

The total conductance, including stomatal and leaf boundary layer conductance to water vapor is calculated using a formula:

$$TCond = Tr(1000 - (W_1 + W_s)/2)/(W_1 - W_s),$$

where: W_1 – concentration of water vapor within the leaf, W_s – concentration of water vapor within the leaf chamber.

Boundary layer conductance is a default variable for a specific measurement, and is determined using a formula:

$$TBCond = Table(s, v_f),$$

where: s – leaf area, which fills the chamber, v_f – fan speed.

Measurements were made on the youngest fully expanded leaves between 10 a.m. and 14 p.m. The photosynthetic parameters were fixed automatically every 20 s, each measurement was performed for 15 min, and the stability of the system was carefully observed and was higher than 2/3.

Assay of lipid peroxidation. Concentration of malondialdehyde (MDA), the end-product of lipid peroxidation, was used as a biomarker of membrane oxidative damage. MDA content was determined by reaction with thiobarbituric acid (TBA) giving the pink-colour compound after heating. The sample of leaf tissue was homogenized with Tris-HCl buffer solution, pH 7.4, containing 1.5% of polyvinylpyrrolidone (PVPP) and centrifuged at 10 000 g for 30 min at 4°C. Equal amounts of tissue extract and 0.5% TBA in 20% trichloroacetic acid (TCA) (w/v) were mixed and heated at 95°C for 30 min. Reaction was stopped by transferring tubes on ice. After centrifugation of the reaction mixture at 10 000 g for 15 min, absorbance of the colored supernatant was measured at 532 nm and corrected for unspecific turbidity by subtracting the value of absorbance at 600 nm. The concentration of MDA was expressed in nmol g^{-1} fresh weight using an extinction coefficient of $155 \text{ mM}^{-1}\text{cm}^{-1}$ (Buege, Aust, 1978).

Statistical analyses. Software *Statistica 6* was applied for statistical analysis and *MS Excel 2010* for presentation of the data. The mean values are presented with the SE of three replicates.

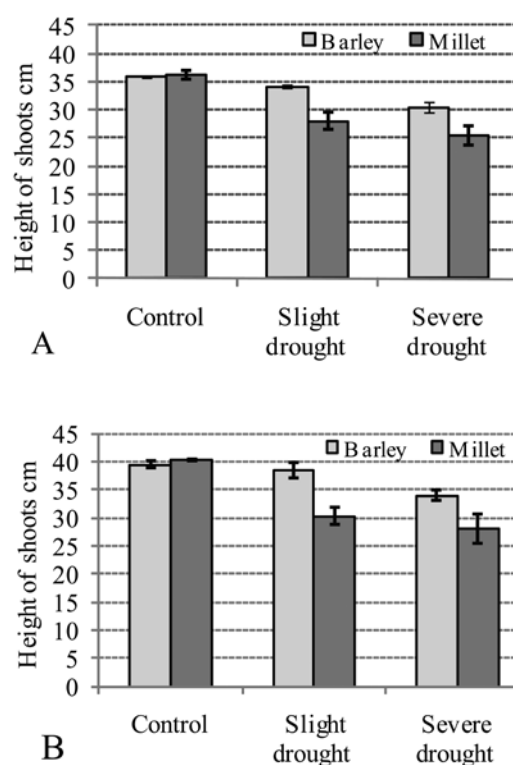
Results and discussion

The drought resistance of barley and millet plants was investigated in this research. The impact of water deficit on growth, photosynthetic apparatus, and gas exchange and especially on membrane oxidative damage varied highly between the plant species subjected to relatively slight (20% reduction of watering) or severe (70% reduction of watering) drought.

Plant height and growth of biomass. The height and the growth of fresh and dry biomass of shoots were

measured at the end of the drought treatment and after the period of recovery. The most obvious reduction was detected in the shoot height, whereas the accumulation of dry biomass was the least affected by water deficit. This was observed in both plant species; however, the growth of the barley plants was less affected by drought treatment than the millet plants.

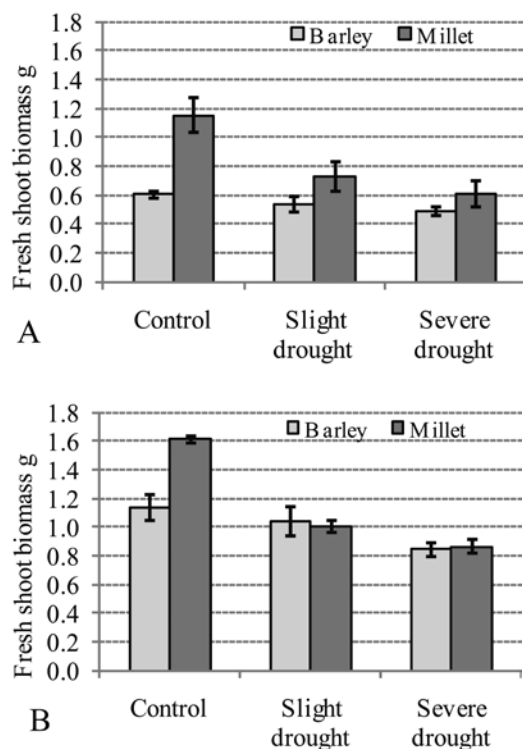
The height of shoots is presented in Figure 1. The height of the barley shoots was inhibited by 5.0% and 15.4%, when watering was reduced by 20% or 70%, respectively. It decreased from 35.9 cm (untreated control) to 34.1 cm or 30.3 cm, respectively for slight or severe drought. The more pronounced reduction was detected in the millet plants: the height decreased by 18.7% and 28.9% (from 34.4 cm to 31.0 cm and 25.5 cm) respectively for the slight and severe drought (Fig. 1 A).



Notes. A – height after drought treatment, B – height after the period of recovery. The whiskers indicate the standard errors of three replicates ($p < 0.05$).

Figure 1. The impact of drought on the shoot height of barley and millet plants

The average fresh shoot biomass of control barley plant was 0.60 g. The reduction by 11.9% (0.54 g per plant) was detected when the watering was reduced by 20%. In the case of severe drought, the fresh biomass decreased by 18.9% (0.49 g per plant). The inhibition of biomass growth was higher in the millet plants (Fig. 2 A). The fresh biomass of the millet decreased from 1.05 g per plant to 0.73 g (30.3%) and 0.61 g per plant (42.0%) after slight or severe drought, respectively. Both the reduction in the height and in the fresh biomass of plants' shoots was statistically significant ($p < 0.05$) (Figs 1 A and 2 A).



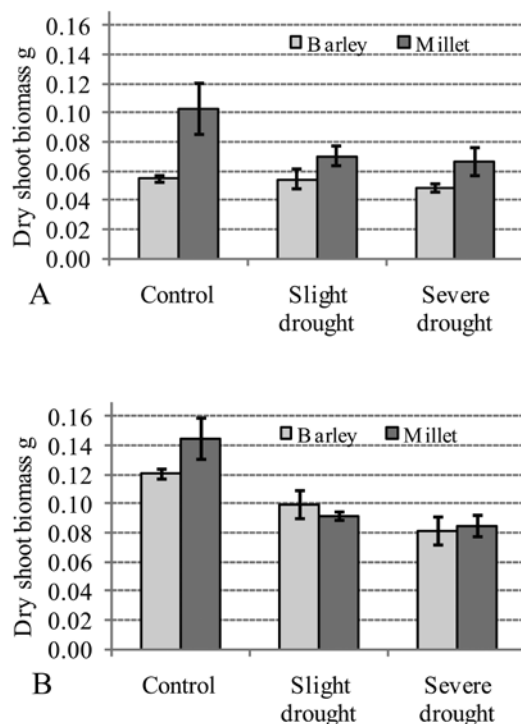
Notes. A – biomass after drought treatment, B – biomass after the period of recovery. The whiskers indicate the standard errors of three replicates ($p < 0.05$).

Figure 2. The impact of drought on the fresh shoot biomass of barley and millet plants

The accumulation of dry shoot biomass of barley was affected statistically significantly by severe water deficit only. The reduction of dry weight by 12.7% was detected: from 0.055 g of control plant to 0.048 g of drought-treated plant. Higher and statistically significant reduction was observed in the millet after both drought treatments. Accumulation of dry biomass was reduced by 12.5% and 17.7% after slight and severe drought, respectively (Fig. 3 A).

When the drought treatment was terminated, the plants were re-watered and the recovery of growth was evaluated after one week. The barley plants were more able to recover in comparison to the millet plants, especially after the slight drought. Both the height and the fresh biomass of barley shoots achieved the control level during the period of re-watering in the case of slight drought (Figs 1 B and 2 B). When barley was subjected to severe drought, the plant height, as well as the fresh and dry biomass of shoots increased during the period of re-watering; however it remained lower than control. In contrast to the barley plants, the growth of the millet was not able to recover completely even after slight drought. The shoot biomass of the millet plants increased when both slight or severe drought were terminated, however, it remained lower as compared with the control plants (Figs 2 and 3).

Drought impact on growth of both C3 and C4 plants is an object of many researches (Liu, Stutzel, 2004; Nayyar, Gupta, 2006; Šliogerytė et al., 2009). Growth rate of C4 plants were found to be less affect-

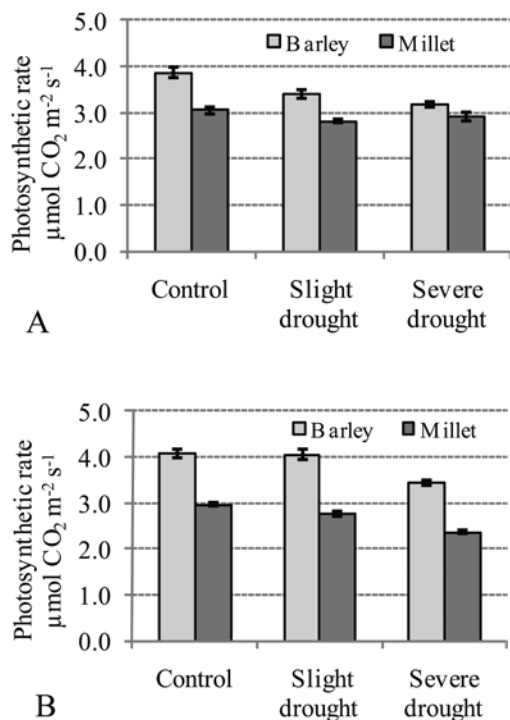


Notes. A – biomass after drought treatment, B – biomass after the period of recovery. The whiskers indicate the standard errors of three replicates ($p < 0.05$).

Figure 3. The impact of drought on dry shoot biomass of barley and millet plants

ed than C3 plants, when *Zea mays* (C4) were compared with *Triticum aestivum* (C3) (Nayyar, Gupta, 2006), and *Cleome gynandra* (C4) with *Cleome spinosa* (C3) (Uzilday et al., 2011). The recovery of drought treated plants has been investigated by lower extent. Šliogerytė et al. (2009) detected, that drought-treated maize was able to recover completely during one week of re-watering after 10 days of drought. In contrast to the previous researches, the growth was more impaired in the C4 crop common millet than in spring barley, moreover, the barley plants were more able to recover. Ripley et al. (2007) also demonstrated lower drought resistance of C4 subspecies of *Alloteropsis semialata* as compared to C3 subspecies of the same species. This paradox might be explained by high metabolic limitations, resulting from biophysical and biochemical processes that are to some degree resolved in C3 plants. Although the reasons why metabolic limitations, such as reduction in metabolites' pool sizes and enzymes activities, as well as osmotic effects on plasmodesmatal functions, etc., are greater in C4 than C3 leaves are unclear, it can result in reduction of C4 pathway's advantages, in spite of higher water use efficiency (Ripley et al., 2007). On the other hand, the genotypic differences are important when different plant species are compared in their stress tolerance. For example, C4 plants not only use water more efficiently, but they require higher temperature and light intensity than C3 plants (Larcher, 1995). Besides, drought treatment was applied during plant tillering, and millet is the most sensitive to water shortage during this period (Svirskis, 2009 b).

Photosynthetic apparatus. The drought effect on the photosynthetic apparatus was evaluated according to the photosynthetic rate (CO_2 assimilation), the gas exchange parameters (stomatal conductance, intercellular CO_2 concentration and transpiration rate) and the WUE. The changes in these parameters were dependent on both drought intensity and the plant species.



Notes. A – photosynthesis after drought treatment, B – photosynthesis after the period of recovery. The whiskers indicate the standard errors of three replicates ($p < 0.05$).

Figure 4. Photosynthetic rate of slight and severe drought-treated barley and millet plants

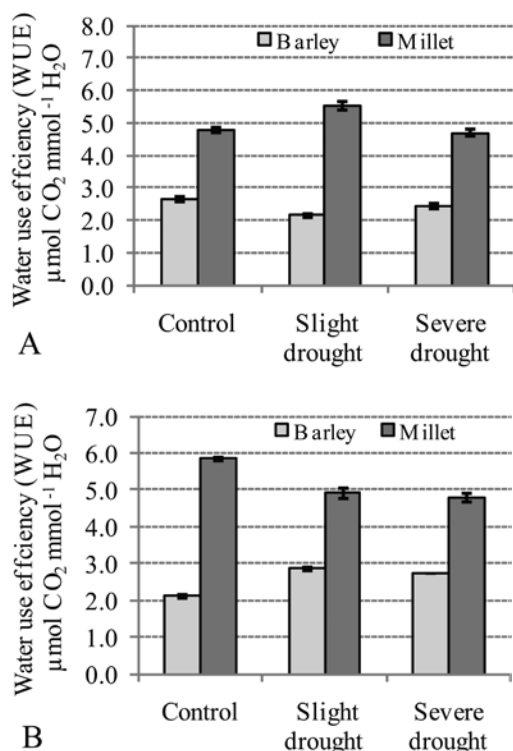
Higher photosynthetic rate was observed in the untreated barley plants compared to the millet plants. However, the CO_2 assimilation of barley was much more seriously affected by the water deficit (Fig. 4 A). The decline by 12.0% and 17.7% of control level was detected when watering was reduced by 20% and 70%, respectively, whereas the photosynthetic rate of the slight drought-treated millet reduced by 7.6%. Even lower and statistically insignificant reduction (4.6% of control) was detected in the severe drought-treated millet plants. On the other hand, the photosynthesis of barley was more able to recover after drought. When the plants were re-watered after slight water deficit, the photosynthetic rate of the barley plants recovered completely, whereas it remained diminished in the millet plants. Moreover, CO_2 assimilation sharply decreased during the re-watering period in the millet plants exposed to severe drought and did not change in the barley plants (Fig. 4 B).

The parameters of gas exchange are given in Table 1 and the WUE is presented in Figure 5. The stomatal conductance, intercellular CO_2 concentration and transpiration rate were considerably lower even in the untreated millet plants. However, the effects of slight and severe drought in the investigated plant species were highly divergent. In the case of millet, the slight shortage of watering enhanced the stomatal closure that was followed by the suppression of gas exchange (detected by reduced transpiration rate and CO_2 concentration) (Table) and improvement in the water use efficiency (Fig. 5 A). The effect of severe drought was less intense: the reduction of stomatal conductance and intercellular CO_2 concentration was lower; besides, no statistical significant impact on transpiration rate and WUE was detected. An opposite effect was observed in the barley plants: slight drought increased stomatal conductance, intercellular CO_2 concentration and transpiration rate, and, as a consequence, WUE was reduced. Stomatal conductance and transpiration rate of barley was diminished due to severe water deficit; nevertheless, WUE remained lower than control. In contrast, WUE reduced in the millet and increased in the barley plants when drought was terminated.

Table. Drought impact on the characteristics of the photosynthetic apparatus

Treatment	Stomatal conductance	Transpiration rate	Intercellular CO_2 concentration
	$\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$	$\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$	$\mu\text{mol CO}_2 \text{ mol}^{-1}$ of air
After drought			
Barley			
Control	0.058 ± 0.001 a	1.467 ± 0.018 a	264.875 ± 2.863 a
Slight drought	0.061 ± 0.001 b	1.594 ± 0.018 b	281.455 ± 2.407 b
Severe drought	0.049 ± 0.002 c	1.351 ± 0.044 c	265.184 ± 2.986 a
Millet			
Control	0.025 ± 0.001 d	0.642 ± 0.019 d	184.214 ± 3.437 c
Slight drought	0.018 ± 0.0003 e	0.516 ± 0.010 e	130.019 ± 6.097 d
Severe drought	0.021 ± 0.0003 f	0.622 ± 0.010 d	163.154 ± 5.007 e
After recovery			
Barley			
Control	0.079 ± 0.001 g	1.934 ± 0.021 e	294.225 ± 1.085 f
Slight drought	0.056 ± 0.002 a	1.424 ± 0.043 ac	259.417 ± 2.530 a
Severe drought	0.048 ± 0.0004 c	1.273 ± 0.010 f	264.643 ± 0.939 a
Millet			
Control	0.019 ± 0.0001 h	0.509 ± 0.003 e	127.000 ± 2.446 d
Slight drought	0.021 ± 0.0004 f	0.574 ± 0.013 g	162.612 ± 5.958 e
Severe drought	0.017 ± 0.0001 i	0.494 ± 0.002 h	161.455 ± 4.230 e

Notes. Data are the means of three replicates \pm standard error. Letters indicate significant differences ($p < 0.05$) between the means.



Notes. A – WUE after drought treatment, B – WUE after the period of recovery. The whiskers indicate the standard errors of three replicates ($p < 0.05$).

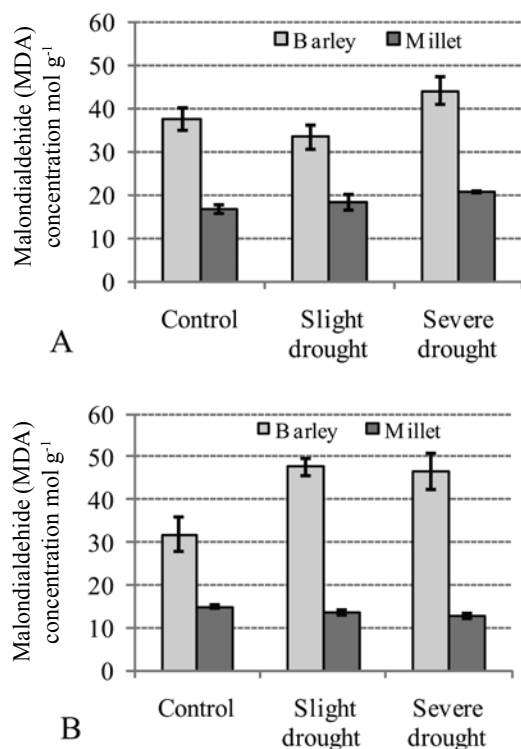
Figure 5. Water use efficiency (WUE) of slight and severe drought-treated barley and millet plants

Photosynthesis is one of the main research objects in many studies of water deficit impact on plants (Nagy et al., 1998; Griffiths, Parry, 2002; Lawlor, Cornic, 2002; Ripley et al., 2007; Ghannoum, 2009). The results of different researches are rather controversial, showing both higher and lower resistance of C4 plants to water deficit. The results of experiment with sorghum and sunflower (Zhang, Kirkham, 1995) agree with our study: higher resistance of photosynthesis to drought was detected in sorghum (C4), most probably due to higher stomatal resistance and higher leaf water potential. Stomatal limitation is generally considered to be the main factor of drought damage on plant photosynthetic apparatus (Zhang, Kirkham, 1995; Cornic, 2000; Medrano et al., 2002). High stomatal resistance allows low transpiration rate and high water use efficiency, which is generally accepted to be responsible for C4 plants' tolerance to drought (Nagy et al., 1998; Gong et al., 2006). The CO₂ concentrating mechanism of C4 metabolism allows prolonged stomatal closure, whereas C3 plants suffer the lack of CO₂, giving rise in the photorespiration rate and reduced photosynthetic efficiency (Zhang, Kirkham, 1995; Noctor et al., 2002). On the other hand, an advantage of prolonged stomatal closure can be outweighed by metabolic limitations, mostly by reduced RuBP synthesis, especially in the case of severe drought (Lawlor, Cornic, 2002). Moreover, multiple adaptive responses at the whole plant level usually occur both in C3 and C4 plants as soon as water deficit begins, including alteration in reserves allocation, hormonal balance, C and N metabolism, dissipation of excitation energy, etc. In this regard, down-

regulation of photosynthesis and photorespiration in C3 plants serve as adjustment of photosynthetic machinery to limited resources (Chaves et al., 2002; Ghannoum, 2009). According to Noctor et al. (2002) photorespiration, which occurs in high rates of C3 plants' leaves, plays a crucial role in C3 plants acclimation to drought. It serves as alternative electron sink when CO₂ is limited due to prolonged stomatal closure, and prevents the photosynthetic electron chain from photoinhibition. Besides, higher metabolic limitations of C4 compared to C3 photosynthesis was demonstrated by Ripley et al. (2007) and could partly explain the better capacity of barley plants to recover after drought treatment, observed in our study. To date, C4 plants ability to recover after re-watering is still poorly understood and it should be pointed out, that photosynthetic rate of millet plants were less able to recover.

Lipid peroxidation. Membrane oxidative damage was evaluated according to the increment of malondialdehyde (MDA) concentration in leaves, indicating the intensity of lipid peroxidation. Considerably higher MDA concentration was found in the control barley plants (37.5 nmol g⁻¹), as compared to the untreated millet plants (16.6 nmol g⁻¹). The slight drought had no statistically significant effect on lipid peroxidation, but it should be pointed out that MDA concentration tended to decrease in the barley leaves and to increase in the millet leaves by approximately 10% (Fig. 6 A). When plants were subjected to severe drought, the concentration of MDA increased by 17.1% (up to 43.9 nmol g⁻¹) and by 24.7% (up to 20.7 nmol g⁻¹) in the leaves of barley and millet, respectively. However, the period of re-watering had a completely different impact on lipid peroxidation in these plants. The membrane oxidative damage reduced considerably in the millet leaves, when drought stress was terminated. Moreover, the extent of reduction was in line with the drought intensity (the reduction by 8.9% and 14.5% was detected when plants were able to recover after slight or severe stress, respectively). On the other hand, the substantial increase in the MDA concentration up to 47.5 nmol g⁻¹ (50.1% from control level) was observed in the leaves of barley plants which were re-watered after the slight drought. In the case of recovery after the severe drought, the MDA concentration remained unchanged (46.5 nmol g⁻¹; i.e. 46.7% higher than control).

Oxidative stress is a non-specific impact of water deficit: the synthesis of reactive oxygen species (ROS) occurs in large extent during desiccation of plant tissues (Xoconistle-Cazares et al., 2010). ROS are produced in a number of ways in plant cells, but photosynthesis plays a predominant role in ROS production. 70% of hydrogen peroxide is synthesized in peroxisomes during photorespiration, and 10% of electrons of PSII reduce oxygen to superoxide during Mehler reaction (Noctor et al., 2002). Apart from this, singlet oxygen is produced when atmospheric oxygen gets energy from excited chlorophyll. Finally, highly reactive hydroxyl radical can be formed in the presence of superoxide and H₂O₂. Thus, ROS synthesis is activated when metabolism and especially photosynthesis are triggered (Dat et al., 2000). ROS production and oxidative damage are expected to be lower in C4 plants, mostly due to an absence of photorespiration (Uzilday et al., 2011). This was proven by our study, since lipid peroxidation was considerably lower in the C4



Notes. A – MDA after drought treatment, B – MDA after the period of recovery. The whiskers indicate the standard errors of three replicates ($p < 0.05$).

Figure 6. The intensity of lipid peroxidation in the slight or severe drought-treated barley and millet plants

plants. Although relative increase in MDA content was higher in the millet, this most probably did not damage plant tissues and, in contrast, induced adaptive responses, because lipid peroxidation sharply reduced during recovery. Whereas oxidative damage further increased in barley leaves, even after drought stress had been terminated. Similarly to our study, considerably lower membrane oxidative damage was detected in maize (C3) as compared to wheat (C4), due to higher antioxidative system capacity. Higher concentration of non-enzymatic antioxidants ascorbic acid and glutathione, as well as higher activity of antioxidative enzymes was detected in maize (Nayar, Gupta, 2006). Similar results were obtained when comparing sorghum and sunflower (Zhang, Kirkham, 1995). Stable level of H_2O_2 and MDA concentration was also detected in C4 plants *C. gynandra* despite relatively low antioxidative capacity and this phenomenon was explained by low photorespiration in C4 leaves (Uzilday et al., 2011). On the other hand, photorespiration plays a multiple role in plants. Apart from its protective role in photosynthetic process, it regulates redox state of the cell and can indirectly increase plant resistance to various stresses. H_2O_2 , which is produced mostly during photorespiration, participates in stress signal transduction and the control of gene expression (Noctor et al., 2002). Enhanced synthesis of H_2O_2 in peroxisomes of barley could be responsible for their better recovery after drought, since hydrogen peroxide is proved to be one of the most important molecules in signal transduction, and to elicit adaptive responses in plants.

Conclusions

1. The C4 crop common millet was more drought-resistant only according to several and not to all investigated indexes. The growth of millet was more markedly affected by drought stress, whereas the photosynthetic apparatus of the millet was more resistant to water deficit. On the other hand, the barley plants were more able to recover: their growth and photosynthetic rate achieved the control level during one week of re-watering.

2. The stomatal conductance, intercellular CO_2 concentration and transpiration rate were considerably lower in the millet, and the difference was even more pronounced after the slight drought.

3. Low transpiration rate determined considerably higher water use efficiency of the control millet plants; besides, it was sharply increased by slight drought in millet, and reduced in barley. However, the water use efficiency (WUE) reduced in the millet and increased in the barley plants when drought was terminated.

4. Considerably higher lipid peroxidation was found in the control barley plants as compared to the untreated millet plants; however, malondialdehyde (MDA) concentration increased more in the millet after severe drought. In contrast, membrane oxidative damage reduced in millet and sharply increased in barley during the period of re-watering, possibly due to enhanced photorespiration in the C3 plants.

5. Our results suggest that the advantages of the C4 photosynthetic pathway could lead to higher drought resistance due to lower oxidative stress and higher WUE. On the other hand, the contribution of non-stomatal and redox-independent limitations of growth remains to be elucidated.

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Atsparumo sausrai skirtumai tarp C3 (*Hordeum vulgare* L.) ir C4 (*Panicum miliaceum* L.) augalų

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Santrauka

C4 metabolizmas yra pranašesnis už C3 metabolizmą sauso klimato sąlygomis dėl CO₂ koncentracijos mechanizmo, kuris ląsteles apsaugo nuo CO₂ trūkumo ir žiotelėms leidžia ilgesnį laiką būti užsivėrusioms. Tačiau sausras sukelti metabolizmo sutrikimai gali viršyti užsivėrusių žiotelių nulemtą atsparumą. Tyrimų tikslas – nustatyti atsparumo sausrai skirtumus tarp C3 augalo vasarinio miežio (*Hordeum vulgare* L.) ir C4 augalo paprastosios soros (*Panicum miliaceum* L.), vertinant sąlygiškai silpnos ir stiprios sausras poveikį augalų augimui, fotosintezės intensyvumui, dujų apykaitai bei membranų lipidų peroksidacijai. Sorų augimas buvo stipriau slopinamas nei miežių: atitinkamai silpna ir stipria sausra paveiktų miežių aukštis sumažėjo 5,0 ir 15,4 %, sorų – 18,7 ir 28,9 %. Augalų augimui silpna sausra neturėjo esminės įtakos, o stipri sausra miežių sausą biomasę sumažino 12,7 %, sorų – 17,7 %. Kita vertus, sorų fotosintezė buvo atsparesnė vandens trūkumui. Be to, soroms buvo būdinga gerokai mažesnis žiotelių pralaidumas, transpiracija, tarpląstelinė CO₂ koncentracija bei lipidų peroksidacija ir didesnis vandens naudojimo efektyvumas, palyginti su miežiais. Miežiai pasižymėjo geresne geba atsigaivinti po sausras poveikio: augalus paveikus silpna sausra, jų augimas ir fotosintezė pasiekė ikikontrolinį lygį. Tyrimų rezultatai parodė, kad C4 augalų atsparumas sausrai gali būti nulemtas silpnosios oksidacinio streso ir didelio vandens naudojimo efektyvumo. Kita vertus, metabolizmo sutrikimų, tiesiogiai nesusijusių su žiotelių laidumu, fotokvėpavimu ir oksidaciniu stresu, tačiau lemiančių C4 augalų augimo slopinimą, priežastys yra neaiškios; siekiant įrodyti paprastosios soros pritaikomumą augini šiltėjančio klimato sąlygomis, šias priežastis vertėtų nustatyti atliekant papildomus tyrimus.

Reikšminiai žodžiai: *Hordeum vulgare*, *Panicum miliaceum* L., sausra, C4 metabolizmas, oksidacinis stresas.