The response of antioxidative defence system of spring barley (*Hordeum vulgare* L.) and barnyard grass (*Echinochloa crus-galli* L) under elevated CO_2 and temperature

Miškelytė D.[1*], Dikšaitytė A[1]., Žaltauskaitė[1] J., Januškaitienė[1] I., Kacienė[1] G., Sujetovienė[1] G., Juknys[1] R., Sakalauskienė[2] S., Miliauskienė[2] J.

[1]*Vytautas Magnus University, Vileikos 8, Kaunas, Lithuania*

[2]*Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry Kauno street 30, LT-54333, Babtai, Kaunas distr., Lithuania*

*corresponding author: e-mail: diana.miskelyte@vdu.lt

Abstract The major components of climate change include warmer temperature and elevated atmospheric carbon dioxide (CO_2) concentrations. Agricultural yields strongly depend on crop competitiveness with weeds. Climate change will have obvious consequences for crop yields as any differential response between crops and weeds to changing climate will alter weed-crop interaction and potential crop yield losses. As C3 and C4 plant species respond differently both to rising CO_2 and rising temperature, this may alter crops ability to compete with C4 weeds. The aim of this study was to investigate the response of antioxidative defence system of spring barley (*Hordeum vulgare* L.) and barnyard grass (*Echinochloa crus-galli* L.) under future climate conditions. Two climate scenarios were investigated: current climate (21 °C, 400 ppm CO_2) and future climate (25 °C, 800 ppm CO_2). The plants were grown in microcosms: spring barley in competition with barnyard grass. The growth and response of antioxidative defence system were evaluated. Antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were measured. Oxidative stress parameters, such as the concentrations of malondialdehyde were determined. Our results indicated that spring barley and barnyard grass responded in different manner to future climate conditions. It was observed that barnyard grass could become more competitive with barley under future climate conditions.

Keywords: Spring barley, barnyard grass, climate change, antioxidative response

1. Introduction

Global climate is changing and it is affecting our environment in a different ways. Increases in atmospheric CO_2 concentrations suggest a doubling of current global values by the end of the 21st century. Increased CO_2 concentration may alter agricultural productivity by differentially affecting the physiology, biochemistry and growth of crops and weeds. Based on the different C3 and C4 plants photosynthetic pathway, it is anticipated that C3 crops may be favored over C4 weeds as atmospheric CO_2 increases (Valerio et al., 2013). Many experiments and most reviews had been done about weed competition and changes in interaction under elevated CO_2 concentration (Ziska, 2000, 2001; Alberto et al., 1996; Patterson et al., 1984). Anyway, crop–weed interactions may vary significantly by region, depending on local soil, temperature, etc. In addition, it seems likely that future temperature will increase as well as atmospheric CO_2 (IPCC, 2007) and that temperature could also be a significant factor in the determination of C3 crop, C4 weed responses to elevated CO_2 (Alberto et al., 1996). Effects of elevated temperature impact on grasslands have been studied to some extend. Most of the studies have been done with monocultures, growing different plant species separately. In addition, many studies of changes in competitiveness in a changing climate were focused on whole-plant, leaf level and physiological measurements and less frequently on the underlying molecular changes. Moreover, it is also known that changing environmental conditions and stresses commonly induce alterations in plant metabolism and accumulation of ROS. Although ROS, in particular H_2O_2, possibly plays a role as a signaling molecule (Baxter et al., 2014), overproduction leads to damaging effects on lipids, proteins and nucleic acids (Gilt et al., 2010). Again, numerous studies had been done about elevated CO_2, temperature (heat waves) and abiotic stress impact on oxidative stress (AbdElgawad et al., 2014; Farfan - Vignolo et al., 2012). But it is lack of the studies about how the complex of predicted future climate conditions (25 °C, 800 ppm CO_2) in absent of severe abiotic stress would affect competitiveness

between weed and crop and molecular processes of the plant. Objective of this study was to evaluate spring barley (*Hordeum vulgaris* L.) and barnyard grass (*Echinochloa crus-galli* L.) growth and molecular respond to elevated climate conditions when two different species of the plants are grown in microcosm.

2. Materials and methods

2.1. Plant materials and growing conditions

Experiments were conducted in two closed, controlled environment plant growth chambers, located at Vytautas Magnus University. Both plant species were grown in 3-liter (21 cm in height and 10.6 cm in diameter) plastic pots in microcosm – mixed culture (9 crops + 6 weeds per pot), filled with a mixture of field soil (the soil was taken from Aleksandras Stulginskis University training farm, Kaunas district), perlite and fine sand (volume ratio 5:3:2). The pre-set values were also identical in each of the chambers for the duration of a 14 h photoperiod (8:00 a.m. to 10:00 p.m.) and the relative air humidity (RH): 60±3% during the day and 80±6% at night. In order to minimize the effects of differences in growing conditions on plant performance within the same growth chamber, each pot was rotated under the same growing condition every day. Plants were watered daily to a level of saturation.

2.2. Treatments and experimental design

Initially, all plants were grown in the control chamber under the conditions of current air temperature of vegetation period and an atmospheric concentration of carbon dioxide – average day/night temperature of 21/14 °C and 400 μmol mol^{-1} of CO_2. Different climate treatments were started when two true leaves or leaf pairs unfolded according to the BBCH growth stage (BBCH is derived from biologische bundesanstalt, bundessortenamt and the chemical industry, the institutions that jointly developed this scale (Meier, 2001)). Plants exposed to future climate (combined elevated CO_2 and temperature) treatment were grown under 800 mmol mol−1 [CO_2] and 25/18°C air temperatures. The duration of treatment was 14 days. All the treatments were run in three replicates. An atmospheric concentration of CO_2 in the chambers were manipulated automatically by controlling the amount of injected CO2 gas and chamber conditioner. The climate program was controlled by the IGSS 9–13175 software.

2.3. Stress parameters

Malondialdehyde (MDA) was measured as an end product of lipid peroxidation using thiobarbituric acid. Tris-HCl buffer with 1.5% (w/v) of PVPP (pH 7.4) was used for MDA extraction. The supernatant was mixed (volume ratio 1:1) with 0.5% thiobarbituric acid, diluted in 20% trichloroacetic acid (w/v). The mixture was heated at 95°C for 30 min. After centrifugation, the absorbance was measured at 532 nm and corrected for unspecific turbidity by subtracting the value of absorbance at 600 nm (Heath et al., 1968; Wu et al. 2003).

2.4. Antioxidant enzymes

Potassium phosphate buffer (pH 7.8, 0.1 M), containing 2 mM dithiothreitol, 0.1 mM EDTA, 0.5% of PEG 4000 and 1% of PVPP was used for proteins and enzymes extraction. Extracts were centrifuged at 14, 000 x g for 15 minutes at 4 °C. Supernatant was filtered through Sephadex G-25 PD10 columns. The soluble protein concentration in the supernatant was determined by the dye-binding method using bovine serum albumin as standard. (Bradford, 1976). For estimation of SOD activity, the reaction mixture, containing potassium phosphate buffer (pH 7.8, 0.1 M), protein extract, 13 μM riboflavin, 13 mM methionine and 63 μM NBT, was incubated for 5 min at 25°C. One unit of SOD was defined as the enzyme activity that inhibited photoreduction of NBT by 50% (Giannopolitis & Ries 1971; Bailly et al. 1996). CAT activity was estimated by measuring the consumption of H_2O_2 at 240 nm. The reaction mixture contained potassium phosphate buffer (pH 7, 50 mM), protein extract and 3.125 mM H_2O_2. The rate of reduction in light absorbance was measured for 30 seconds (Clairbone 1985; Bailly et al. 1996).

2.5. Leaf area measurement

The measurement of leaf area was carried out on the last day of treatment. The leaf areas of all leaves per plant of five plants per treatment were measured with a scanner (CanoScan 4400F, Canon, USA) and then the leaf area of all leaves per plant was determined by GIMP 2.8 software.

2.6. Statystical analysis

Significant differences between samples were determined by using Fisher LSD test and $p<0,05$ was considered to be significant. All the statistical analysis were carried out using STATISTICA software.

3. Results and discussion

3.1.Growth

Spring barley and barnyard grass total dry mass and leaf area are shown in Figure 1. It was observed that

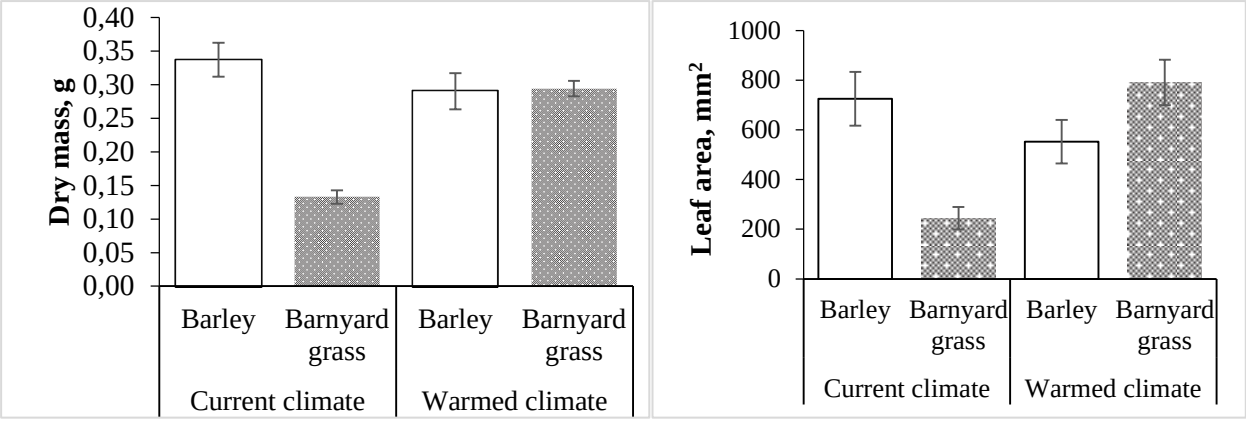


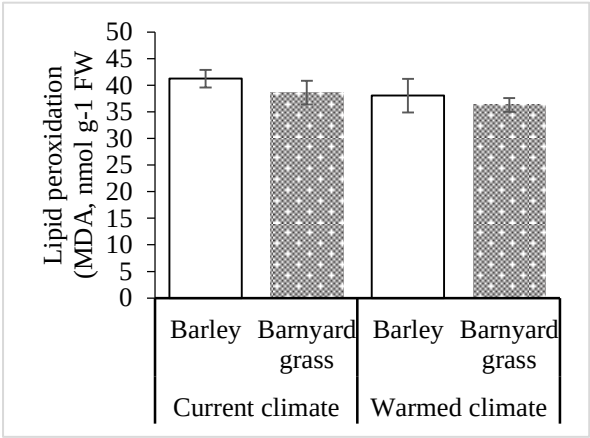
Figure 1. Total dry mass and leaf area of spring barley and barnyard grass under current and elevated climate conditions

elevated CO_2 and temperature conditions have resulted in reduction of all investigated morphological parameters of spring barley in comparison with ambient conditions (21 ^{o} C/400 ppm), however these observations were not statistically significant (p>0,05). The leaf area of spring barley was affected at highest rate by warmed climate – observed reduction was 23, 7 % (p>0, 05). It has been reported that the vegetative growth of C3 plants was enhanced when grown in both elevated CO_2 and temperature (Yoon et al., 2009, Vu, 2005;). However, it was not observed in the current study. Even though reduction in C3 plant spring barley vegetative growth was not statistically significant, but the crop was grown in competitive conditions with the weed (barnyard grass) which could be the main reason of lower performance of spring barley growth.

The growth of barnyard grass reacted to warmed climate in different manner than spring barley. Raised concentration of CO_2 and temperature significantly increased growth of the C4 weed. It was measured that barnyard grass total dry mass and leaf area increased 2.2 (p<0, 05) and 3.2 times (p<0, 05) respectively compared with a control samples. Consistent with the spring barley results, elevated climate conditions affected barnyard grass leaf area at highest rate. Leaf area as well as other morphological parameters are one of the key parameter in competition between plants, which allows plant to compete for the light. The results of this study suggest that spring barley competitive ability decreases and barnyard grass competitive ability increases in a warmed climate conditions. These results are consistent with published results. Yin et al. (2008) and Mahajan et al. (2012) have reported that higher temperatures may increase competitive ability of C4 plant.

3.2 Lipid peroxidation

It has been reported (Farfan – Vignolo et al., 2012) that warmed climate alone did not cause significant lipid peroxidation. This study supports reported results.



Determined malondialdehyde (MDA) content is shown in Figure 2. Future climate did not affect lipid peroxidation. Slight but not significant (p>0, 05) reduction in MDA content was observed in both spring barley and barnyard grass at warmed climate, by 7,7 and 6 % respectively.

Figure 2. Lipid peroxidation in spring barley and barnyard grass at different climate conditions

3.3. ROS scavenging enzymes

Measured SOD and CAT activities are shown in Figure 3. Elevated CO_2 and temperature had no statistically significant impact on activity of antioxidants in spring barley leaf tissues. 55 % reduction (p>0,05) of SOD activity was observed when spring barley was grown in elevated climate. In contrast, CAT activity was enhanced by 33 % (p>0.05) in comparison with ambient conditions. Warmed climate exposure resulted in statistically significant reduction of antioxidative defence system (p<0.05) in barnyard grass. It was measured that SOD and CAT activities were reduced by 53 % and 18 % respectively. The reason of this reduction in C4 plant is not well known. Researches had been mostly testing single effects of elevated CO_2,

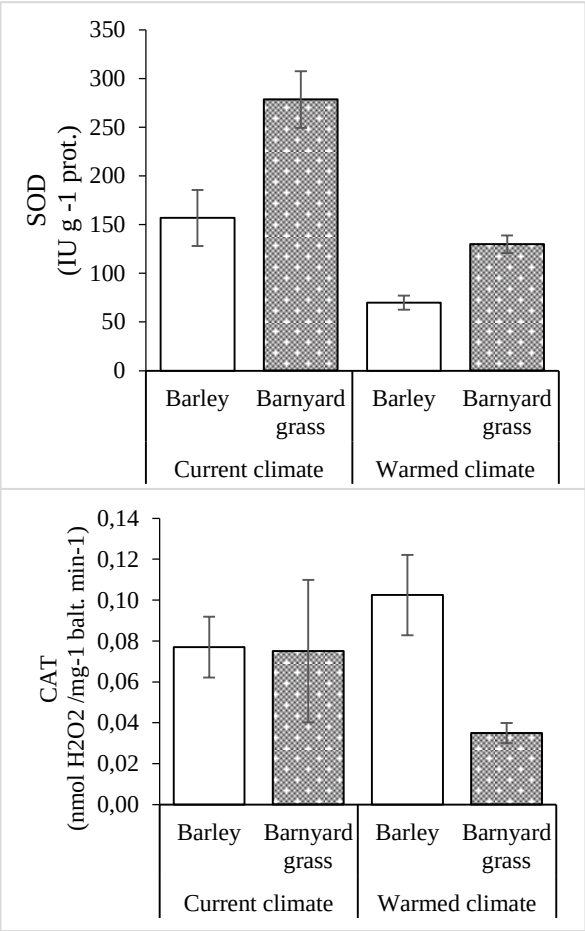


Figure 3. SOD and CAT activity in spring barley and barnyadr grass leaf tissues at different climate conditions

temperature or abiotic stressors impact on oxidative stress. Author reported that elevated CO_2 reduced SOD and CAT activity significantly in all tested species. There is a lack of studies about combined predicted future CO_2 and temperature increase effect on plant metabolism.

4. Conclusion

The results of this study suggest that spring barley competitive ability decreases and barnyard grass competitive ability increases in a warmed climate conditions. Elevated CO2 and temperature resulted in significant ($p<0,05$) reduction of SOD and CAT activities in barnyard grass were reduced by 53 % and 18 % respectively.

5. Acknowledgements

This research was supplemented in the frame of the support from the Research Council of Lithuania (grant number: SIT-8/2015; research project "Composite impact of climate and environmental change on productivity, biological diversity and sustainability of agro-ecosystems" in the frame of National Research Program "Sustainability of Agro, Forest and Water Ecosystems".

References

AbdElgawad H., Farfan-Vignolo E.R., de Vos D.,b, Asard H., (2015), Elevated CO_2 mitigates drought and temperature-induced oxidativestress differently in grasses and legumes, *Plant Science*, **231**, 1–10.

Alberto, A.M., Ziska, L.H., Cervancia, C.R., Manalo, P.A., (1996), The influence of increasing carbon dioxide and temperature on competitive interactions between a C3 crop, rice (Oryza sativa), and a C4 weed (Echinochloa glabrescens), *Aust. J. Plant Physiol*., **23**, 795–802.and Medicago sativa L., *Plant Physiology and Biochemistry*, **59**, 55 – 62.

Baxter A., Mittler R., Suzuki N., (2014), ROS as key players in plant stress signalling, *J.Exp. Bot*, **65,** 1229–1240.

Bradford M., (1976), A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Anal Biochem*, **72**, 248–254.

Farfan-Vignolo E.R., Asard H., (2012), Effect of elevated CO_2 and temperature on the oxidative stress response to drought in Lolium perenne and Medicago sativa L., *Plant Physiology and Biochemistry*, **59**, 55 – 62.

Gill S.S., Tuteja N., (2010), Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants, *Plant Physiol. Biochem*, **48**, 909–930.

Heath R, Packer L., (1968), Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid Peroxidation, *Arch Biochem Biophys*, **125**, 189–198.

IPCC, (2007), Climate Change 2007: Impacts, Adaptation and Vulnerability. IPCC Secretariat, Geneva, Switzerland.

Mahajan G., Singh S., Chauhan B. S., (2012), Impact of climate change on weeds in the rice–wheat cropping system, *Current science*, **102(9)**, 1254–1255.

Meier U., (2001), Growth stages of mono-and dicotyledonous plants. Berlin: Federal Biological Research Centre for Agriculture and Forestry.

Patterson, D.T., Flint, E.P., Beyers, J.L., (1984), Effects of CO_2 enrichment on competition between a C4 weed and a C3 crop, *Weed Sci*, **32**, 101–105.

Valerio M., Tomecek M., Lovelli S., Ziska L.,(2013), Assessing the impact of increasing carbon dioxide and temperature on crop-weed interactions for tomato and a C3 and C4 weed species, *Europ. J. Agronomy*, **50**, 60– 65.

Vu J. C. V., (2005), Acclimation of peanut (*Arachis hypogaea* L.) leaf photosynthesis to elevated growth CO_2 and temperature, *Environment and Experimental Botany*, **53**, 85–95.

Wu F., Zhang G., Dominy P., (2003), Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity, *Environ Exp Bot*., **50**, 67–78.

Yin X., Struik P. C., (2008), Applying modelling experiences from the past to shape crop systems biology: the need to converge crop physiology and functional genomics., *New Phytologist*, **179**, 629 – 642.

Yoon S. T., Hoogenboom G., Flitcroft I., Bannayan M. (2009), Growth and development of cotton (*Gossypium hirsutum*L.) in response to CO_2 enrichment under two different temperature regimes, *Environmental and Experimental Botany*, **67**, 178–187.

Ziska L.H., (2001), Changes in competitive ability between a C4 crop (Sorghum bicolor L.) and a C3 weed, common cocklebur (Xanthium strumarium L.) with elevated carbon dioxide, *Weed Sci*, **49**, 622–627.

Ziska, L.H., (2000), The impact of elevated CO_2 on yield loss from a C3 and C4 weed in field-grown soybean, *Global Change Biol*, **6**, 899–905.

Bailly C, Benamar A, Corbineau F, CoAme D., (1996), Changes in malondialdehyde content and in superoxide dismutase, catalase, and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated ageing, *Physiol Plant*, **97**, 104–110.

Clairbone A., (1985), CRC handbook of methods for oxygen radical research. *Boca Raton: CRC press. Catalase activity*, 283–284.

Giannopolitis CN, Ries SK., (1971), Superoxide dismutases. I. Occurrence in higher plants, *Plant Physiol*, **59**, 309–314.