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

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**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$_2$O$_2$, 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 vulgare 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 CO₂. 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 CO₂ and temperature) treatment were grown under 800 mmol mol⁻¹ [CO₂] 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 CO₂ 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 H₂O₂ at 240 nm. The reaction mixture contained potassium phosphate buffer (pH 7, 50 mM), protein extract and 3.125 mM H₂O₂. 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. Statistical 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
Elevated CO$_2$ and temperature conditions have resulted in reduction of all investigated morphological parameters of spring barley in comparison with ambient conditions (21 °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 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.
temperature or abiotic stressors impact on oxidative stress. Author reported that elevated CO\textsubscript{2} reduced SOD and CAT activity significantly in all tested species. There is a lack of studies about combined predicted future CO\textsubscript{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 CO\textsubscript{2} and temperature resulted in significant (p<0.05) reduction of SOD and CAT activities in barnyard grass were reduced by 53\% and 18\% respectively.

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