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DIFFERENTIAL ANTIOXIDATIVE RESPONSES TO COOPER AND CADMIUM IN ROOTS AND LEAVES OF TRITICALE HEXAPLOID LINES

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ABSTRACT. Triticale (x*Triticosecale* Withmack) hexaploid lines (CCO-1,CCO-2,CCO-3) and its parents wheat and rye exposed to different concentrations of Cu^{2+}, Cd^{2+} and their combination for 14d in aqueous culture were analyzed with reference to the accumulation of biomass and the metal`s effect on some antioxidative enzymes in root and leaves. After treatment fresh mass/dry mass (FM/DM) ratio decreased significantly in the roots in comparison with the leaves. Roots and leaves of investigated lines responded differently to Cu^{2+}, Cd^{2+} and their combination with reference to the induction of enhanced activity of guiacol peroxidase (GP) in wheat and newly created stabilized lines CCO-1. The activity of catalase (CAT) decreased in the leaves and roots of all lines, except CCO-2.

The changes in the accumulated biomass and the activities of antioxidative enzymes showed that newly created triticale hexaploid CCO-2 is more resisitant against heavy metal stress.

KEY WORDS. Triticale, cooper, cadmium, catalase, guiacol peroxidase

INTRODUCTION

Triticale (x*Triticosecale* Withmack) is an amphidiploid of wheat and rye which is used as a model system for investigation of the genomic chromosome and homeoallelic relationships. In its genome are combined some positive qualities of wheat (quality and quantity of grain) and rye (resistance to abiotic and biotic factors).

Secondary hexaploids have basic economic importance and 2D/2R substitution has the highest frequency. Proving the effect of chromosome substitutions and reconstructions on the productivity and resistance to abiotic and biotic factors is important for development of purposeful genetic and selection programs.

The paper is aimed at establishing the responses of morphologically different triticale lines to high concentrations of Cu^{2+}, Cd^{2+} and their combination. The changes in the accumulation of biomass and activity of some antioxidative enzymes (catalase -CAT, guiacol peroxidase -GP) in roots and leaves of newly created triticale lines (CCO-1, CCO-2, CCO-3) and its parents are investigated.

MATERIAL AND METHODS

Plant material. The experiments were conducted on 20-old plants of wheat, rye and hexaploid triticale lines, created at in IG - BAS (CCO-1, CCO-2, CCO-3) grown in a aqueous culture of Hellrigel solution with the addition of A-Z microelements after Hoagland (H e l l r i g e l, 1898) in a phototermostatic chamber with a 12-h photoperiod (PPFD 60 µmol $m^{-2}s^{-1}$, temperature 25±1°C, relative air humidity 50-60%). On the 14th day the plants were treated with Cu^{2+} (as $CuCl_2$) and Cd^{2+} (as $CdCl_2$) were added under following scheme: 1/ Control – full nutrient solution; 2/ 5 g m^{-3} Cu^{2+}; 3/ 10 g m^{-3} Cu^{2+} ; 4/ 15 g m^{-3} Cu^{2+} (threshold toxic concentration); 5/ 3 g m^{-3} Cd^{2+} (threshold toxic concentration) ; 6/ 5 g m^{-3} Cu^{2+} + 3 g m^{-3} Cd^{2+}. The action of investigated concentrations of heavy metals under the above scheme was traced for 14 d.

Physiological and biochemical characteristics. Fresh mass (FM) of leaves and roots were determined after weighting. Dry mass (DM) were determined after drying the plant material at 60°C for 48h until constant weight of samples was reached. For each experiment 4-6 mature leaves were used. In order to prepare crude extracts for determination of catalase (CAT) and guiacol peroxidase (GP) the procedure of A e b y (1984) was used. Two enzymes were assayed spectrophotometrically tracing the changes in absorbance at 240 and 470 nm at 27°C using UV-VIS SPECORD or SPECOL 10. Catalase (EC 1.11.1.6) was assayed according to the procedure of A e b y (1984). Guiacol peroxidase (EC 1.11.1.7) was assayed according to P o l l e et al. (1994). The protein content was determined after L o w r y et al. (1951).

All the results shown were representative of a number of repeated experiments.

RESULTS AND DISCUSSION

The fresh mass/dry mass (FM/DM) ratio in the leaves and roots of treated rye plants increased significantly (Fig. 1c,d). From the newly created triticale lines, CCO-3 is distinguished by increasing FM/DM ratio in the leaves (Fig. 1i). This ratio increased more in the roots of rye plants after treatment with 3g m^{-3} Cd^{2+} (Fig. 1d – variant 5), and slightly decreased for species CCO-1 (Fig. 2f). In general, FM/DM ratio in the roots decreased significantly than leaves after treatment with different concentrations of heavy metals, especially for newly created triticale lines. Our results coincide with other investigations about reduction of root biomass at excess of Cu^{2+} (R o u s e s and H a r r i s o n, 1987; L i d o n and H e n r i g u e s, 1992). The effect of the Cu^{2+} and Cd^{2+} combination on the roots is weaker. Both redox active (Cu, Fe) and non-redox active metal ions (Zn, Cd) are reported to increase lipid peroxidation via reactive oxigen species (ROS) generation in plants (S h a w, 1995; G a l l e g o et al., 1996; C h a o n i et al., 1997). The formation of ROS is prevented by an antioxidant system: low molecular mass antioxidants (ascorbic acid, glutathione, tocopherols), enzymes generating the reduced forms of antioxidants, and ROS-interacting enzymes, such as superoxide dismutase, peroxidases and catalases (B l o k h i n a et al., 2003).

The activity of catalase in the leaves of wheat, rye and newly created triticale lines varied by a different way in dependence of treatment (Fig. 2). The reduction in the activity of CAT in the leaves is observed, excepting CCO-2 line in which this activity increases during treatment (Fig. 2g). This enzyme posses relative substrate specificity and probably it play unimportant role in antioxidative defense of wheat, rye and newly created triticale lines.

The activity of guiacol peroxidase prominently increased in leaves of wheat after treatment with 3 g m^{-3} Cd^{2+} and 3 g m^{-3} Cd^{2+} + 5 g m^{-3} Cu^{2+} (Fig. 3a) and in all variants of treatment of rye (Fig. 3c). Between newly created triticale lines, GP activity showed maximum stimulation in CCO-1 (Fig. 3e). Heavy metal-induced enhancement in GP in the roots is higher for the same line than for CCO-2 and CCO-3 (Fig. 3f,h,j). GP use as a donor of protons guiacol which belongs to the phenolic compounds. Oxidation of phenolics following of the processes of condensation assign significantly role of the enzyme in the lignification processes (Zaprometov, 1977). Our results show increased activities of antioxidative enzymes after treatment with 3 g m^{-3} Cd^{2+} and 3 g m^{-3} Cd^{2+} + 5 g m^{-3} Cu^{2+} suggest that they have some additive function in the mechanism of metal tolerance in triticale. Heavy metal-induced increase in the levels of antioxidative enzymes may present a secondary defensive mechanism against oxidative stress that are not as direct as the primary defensive responses such as phytochelatins and vascular compartmentalization (S a n i t a d e T o p p i and G a b r i e l l i, 1999). Acute concentrations of heavy metals may adversely affect the activity of certain defense enzymes either by inhibiting their synthesis or by their inactivation and down regulation. The significant increase in the activity of GP in the roots than leaves at high concentrations of Cd^{2+} and Cu^{2+} and their combination may be indicative as a rapid signaling response.

The differential responses of antioxidative enzymes after treatment with increasing concentrations of Cu^{2+}, Cd^{2+} and their combination in roots and leaves of parents wheat, rye and newly created triticale lines may be attributed to varied level of ROS generation in two functionally distinct organs of the plants. Metal ions may stimulate the generation of ROS either by direct transfer of electrons in single-electron reactions involving metal cations, or as consequence to metal-inactivated metabolic reactions (D i e t z et al., 1999). Chloroplasts, mitochondria, peroxisomes and plasma membrane–linked electron transport all contribute towards generation of

ROS in leaves. Its generation in the roots are weaker being a non-photosynthetic tissue. The induction of GP activity provide additional defense against metal toxicity and keeps the metabolic activity in roots functional.

CONCLUSION

Cu^{2+}, Cd^{2+} and their combination induced a decrease in the root biomass of wheat, rye and newly created triticale lines CCO-1, CCO-2 and CCO-3. These heavy metal ions caused differential level of oxidative stress in the roots and leaves of triticale plants. The activity of catalase (CAT) decreased in the roots and leaves of all lines, except CCO-2 because it posses relative substrate specificity. Guiacol peroxidase (GP) showed pronounced stimulation in both roots and leaves of triticale lines. Increased activities of antioxidative enzymes after treatment with combination of heavy metal ions suggest some additive function in the mechanism of metal tolerance in triticale plants.

ACKNOWLEDGEMENTS

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ILLUSTRATIONS

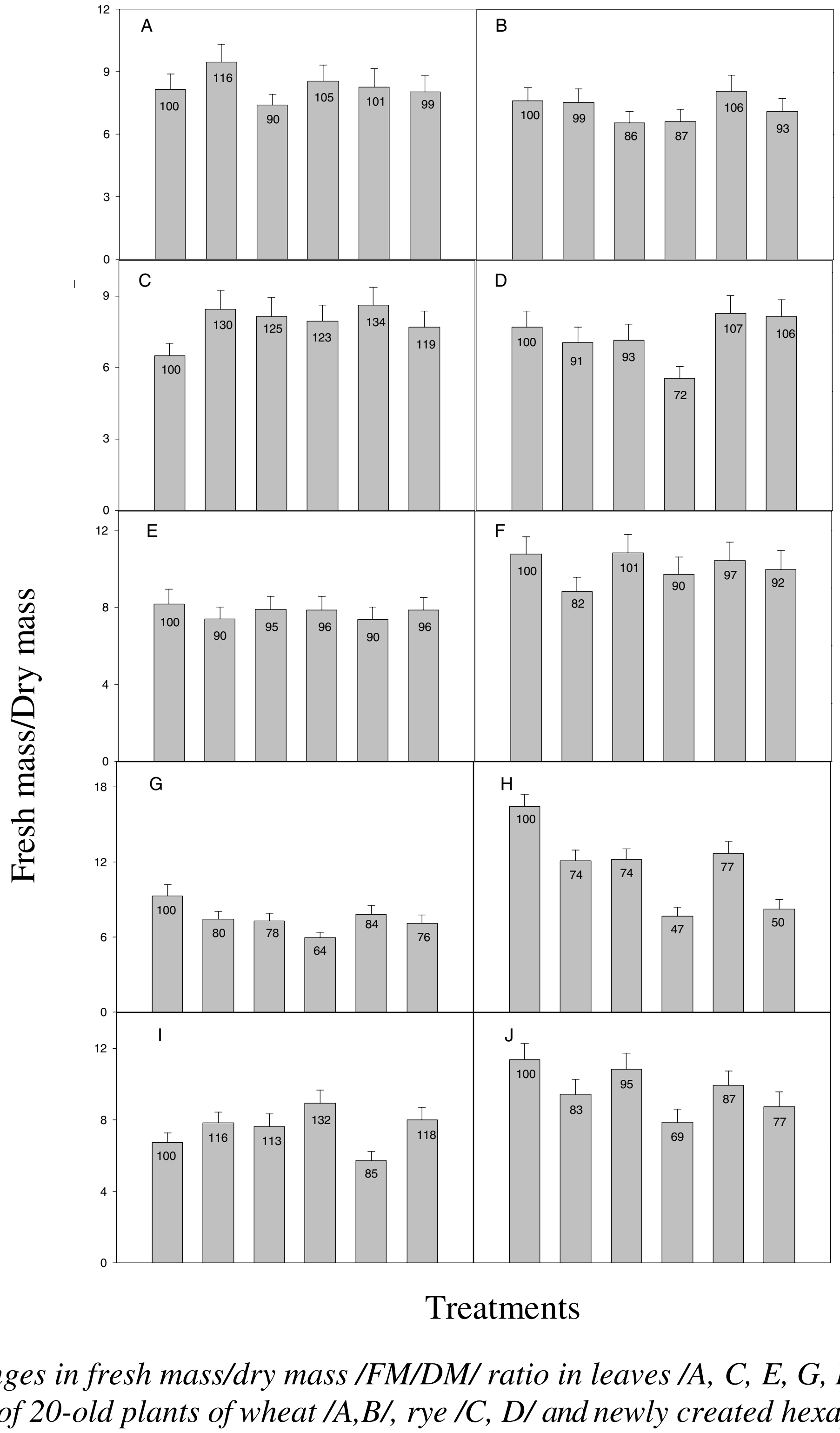

Fig. 1. *Changes in fresh mass/dry mass /FM/DM/ ratio in leaves /A, C, E, G, I/ and roots /B, D, F, H, J/ of 20-old plants of wheat /A,B/, rye /C, D/ and newly created hexaploid triticale lines – CCO-1 /E, F/, CCO-2 /G, H/, CCO-3 /I, J/ treated after following scheme: 1/ Control – full nutrient solution; 2/ 5 g m^{-3} Cu^{2+}; 3/ 10 g m^{-3} Cu^{2+}; 3/ 15 g m^{-3} Cu^{2+}; 4/ 3 g m^{-3} Cd^{2+}; 6/ 3 g m^{-3} Cd^{2+} + 5 g m^{-3} Cu^{2+}. The effect of the action of the heavy metals is registered on the 14-th day. The results are also presented as a percentage of the control. Each mean represents 4-6 independent measurements ±S.D.*

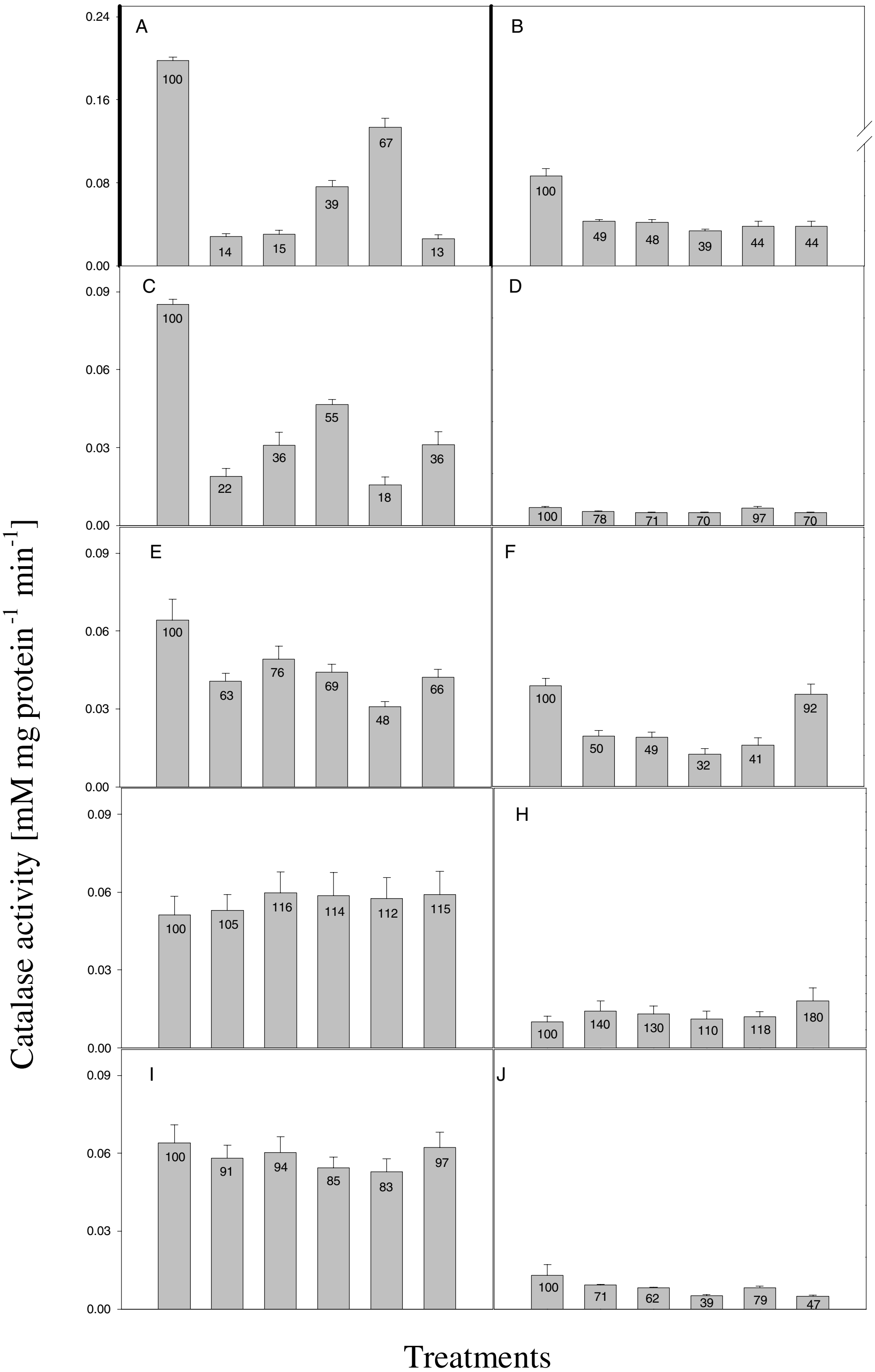

Fig. 2. *Changes in the catalase activity in leaves /A, C, E, G, I/ and roots /B, D, F, H, J/ of wheat /A, B/, rye /C, D/ and newly created triticale lines – CCO-1 /E, F/, CCO-2 /G, H/, CCO-3 /I, J/. The designations of the individual variants of the experiments are the same as in the Fig. 1. Each mean represented 4-6 independent measurements±SD.*

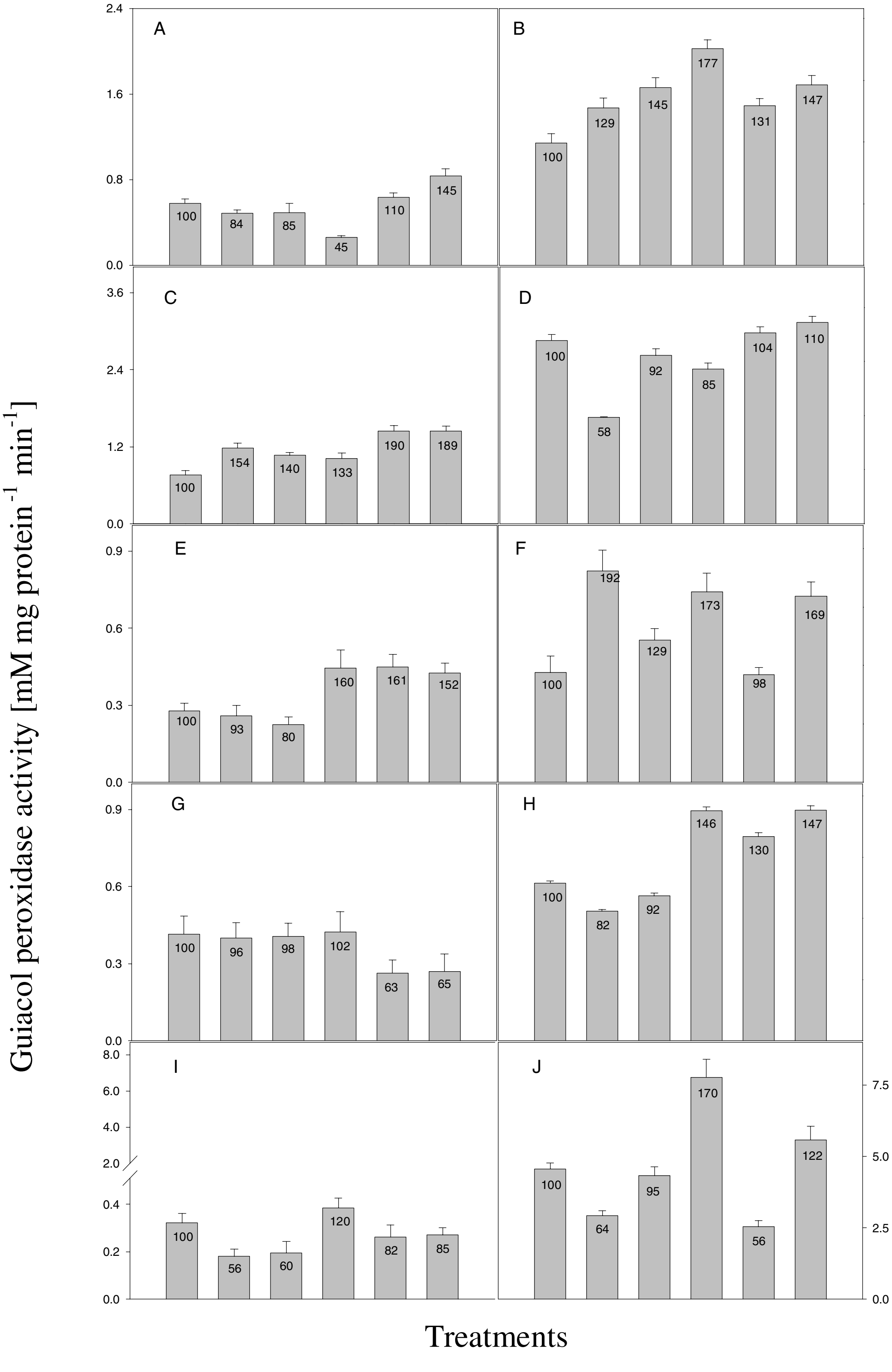

Fig. 3. *Changes in the guiacol peroxidase activity in leaves /A, C, E, G, I/ and roots /B, D, F, H, J/ of wheat /A, B/, rye /C, D/ and newly created triticale lines – CCO-1 /E, F/, CCO-2 /G, H/, CCO-3 /I, J/. The designations of the individual variants of the experiments are the same as in the Fig. 1.*