BOOK OF ABSTRACTS

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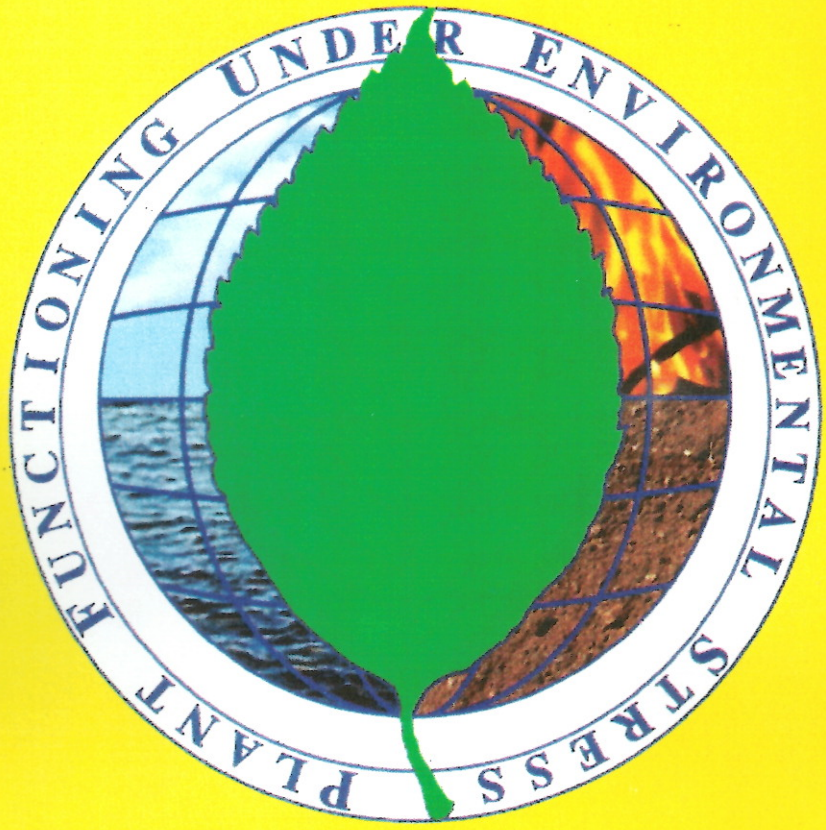

Redox regulation of androgenesis in rye (*Secale cereale* L.)

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Among cereals, rye is one of the most recalcitrant to androgenesis, both in anther (AC) and isolated microspore (ME) cultures. Our earlier investigations revealed that an important role in efficient stress-induced androgenesis is played by oxidative stress and redox-regulating antioxidants **(Żur et al., 2009, 2014).** High activity of enzymatic and non-enzymatic antioxidants eliminates reactive oxygen species (ROS) and sustains proper redox homeostasis. Glutathione (GSH) belongs to the most abundant cell peptides involved in ROS scavenging. We recently reported that optimal glutathione level, reduced/oxidized glutathione ratio (GSH/GSSG) and glutathione enzyme activity reduce oxidative stress and stimulate androgenesis in rye (Zieliński et al. 2015). As glutathione peroxidase (GPX) plays a pivotal role in the protection of cells against oxidative damage, in the present study the effects of N-acetyl-L-cysteine (LNAC, 10 μM) – glutathione precursor, and selenium (50 mM sodium selenate), known as GPX activity modulator, were investigated and related to androgenesis effectiveness. As the balance between reduced/oxidized state of glutathione is controlled by glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione S-transferase (GST), their activity was determined spectrophotometrically.

Three rye genotypes used in the study, provided by Polish breeding companies, differed significantly in response to androgenesis induction as well as in glutathione content catalysed by GPX, GR and GST enzymes. Stress (low temperature, mannitol), antioxidants (GSH, LNAC) and selenium treatments affected the contents of endogenous GSH and oxidized glutathione (GSSG). The treatment of microspores with LNAC stimulated androgenesis induction.

The results indicate that by modifying the level of oxidative stress, recalcitrancy of rye to androgenesis induction could be alleviated. Treatment with GSH, LNAC or selenium affected the endogenous level of glutathione and probably enhanced the defence ability against the stress induced by low temperature spikes pre-treatment. Optimal GSH/GSSG redox ratio could stimulate androgenesis in some lines of rye described as recalcitrant to androgenesis.

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REDOX REGULATION OF ANDROGENESIS IN RYE (*SECALE CEREALE* L.).

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INTRODUCTION

Among cereals, rye is one of the most recalcitrant to androgenesis, both in anther (AC) and isolated microspore (ME) cultures. Our earlier investigations revealed that an important role in efficient stress-induced androgenesis is played by oxidative stress and redox-regulating antioxidants (Żur et al. 2009, 2014). High activity of enzymatic and non-enzymatic antioxidants eliminates reactive oxygen species (ROS) and sustains proper redox homeostasis. Glutathione (GSH) belongs to the most abundant cell peptides involved in ROS scavenging. We recently reported that optimal glutathione level, reduced/oxidized glutathione ratio (GSH/GSSG) and glutathione enzyme activity reduce oxidative stress and stimulate androgenesis in rye (Zieliński et al. 2015). As glutathione peroxidase (GPX) plays a pivotal role in the protection of cells against oxidative damage, in the present study the effects of N-acetyl-L-cysteine (LNAC) – glutathione precursor, and selenium (sodium selenate), known as GPX activity modulator, were investigated and related to androgenesis effectiveness. As the balance between reduced/oxidized state of glutathione is controlled by glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione S-transferase (GST), their activity was determined spectrophotometrically.

MATERIAL & METHODS

Material

Tillers of six rye (*Secale cereale* L.) genotypes (lines: 3, 4, 5, 8, 10, 14), provided by Polish breeding companies. Spikes were collected when the majority of microspores were at mid- to late uni-nucleated stage of development, optimal for androgenesis induction.

Induction of androgenesis by stress pre-treatment

Collected tillers were placed in Hoagland's salt solution (LT) or mannitol with glutathione (Mn_GSH) and stored at 4°C in the dark for 4 weeks. The spikes' pretreatment system was comprised of: control (Hoagland's salt solution) or sodium selenate (SS,50 mM) or N-acetyl-L-cysteine (LNAC, 10 μM).

Anther cultures

Anther culture procedure described by Immonen and Tenhola-Roininen (2003) was used after several modifications. The regeneration of androgenic structures (AS) of 1 mm size was done on modyfied 190-2 medium (Zhuang and Xu 1983; Fig. 1). Tested rye lines were grouped into three classess according to the androgenesis effectiveness: recalcitrant, moderate and responsive. To each group belonged two rye genotypes significantly different in their response to androgenesis induction (highly responsive: 8, 14; moderate: 4,10 and recalcitrant: 3, 5).

Biochemical analyses

Glutathione concentrations and glutathione enzymes activity were measured spectrophotometrically in anthers isolated from pre-treated tillers) at 412 nm or 340 nm, respectivelly (Fig. 2). GSH/(GSH+GSSG) ratio values, obtained by GSH and GSSG levels are the mean ± SE of three experiments repeated in triplicate. Similar, enzymes activity are the mean ± SE of three experiments repeated in triplicate.

Measurement of oxidized (GSSG) and reduced (GSH) glutathione

GSH and GSSG were determined by the method of Knörzer et al. (1996). The absorbance of GSH and GSSG was measured at for 3 min. The activity of glutathione reductase was proportional with glutathione concentrations. The glutathione concentration was calculated from a calibration curve prepared with glutathione solutions of known concentrations.

Glutathione enzymes activity

Glutathione peroxidase (GPX) was determined according to the Hopkins and Tudhope (1973) using tert-butyl hydroperoxide (t-Bu-OOH). Glutathione reductase (GR) activity was measured according to the Klapheck el al. (1990) with NADPH oxidation. Glutathione S-transferase (GST) activity was determined according to the Mauch and Dudler (1993) using 1-chloro-2,4-dinitrobenzene (CDNB).

Statistical analysis

All statistical analyses were performed using STATISTICA package version 13.1 (Stat Soft Inc., USA, 2016). The effect of tested variables, describing glutathione content and enzymes activity, was examined by multi-factor analysis of variance (ANOVA). Post-hoc comparison was conducted with the use of Duncan's multiple range test ($p \leq 0.05$).

Fig. 1. Rye anther cultures.

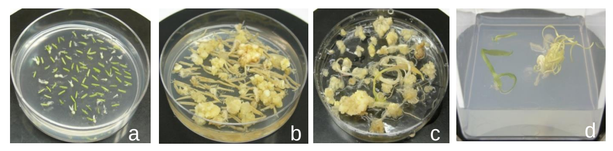

Fig. 1. a. Anthers after isolation from low temperature pre-treated spikes. **b**. Anthers after 6 weeks of culture on induction medium. **c**. Embryo-like structures on regeneration medium. **d**. Green and albino plants in rooting medium.

Fig. 2. The scheme of the experiment.

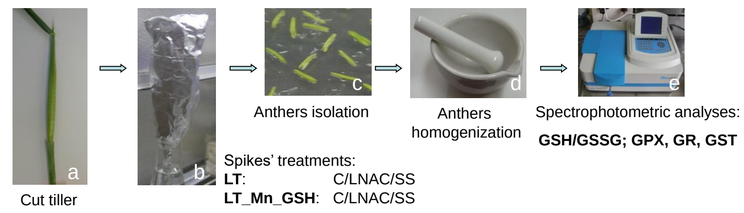

Fig. 2. a. Freshly cut tiller. **b**. Pre-treated spikes. LT, low temperature spikes treatment in Hoagland medium; LT_Mn_GSH low temperature spikes treatment in solution of GSH and Mn. C, control (without additional treatment), LNAC or SS (additional treatments). **c**. Anthers isolated from the pre-treated spikes. **d**. Anthers homogenisation. **e**. Spectrophotometric analyses of GSH/GSSG, GPX, GR, GST.

Fig. 3. The GSH/(GSH+GSSG) ratio.

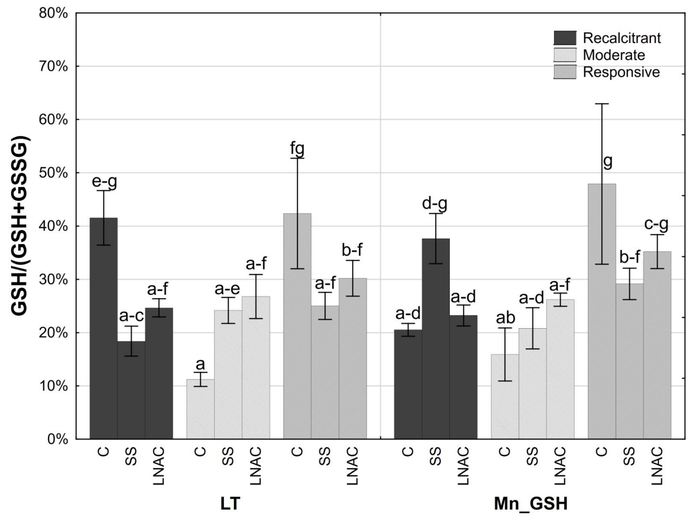

Fig. 3. Effect of SS and LNAC on the redox status expressed as the GSH/(GSH+GSSG) ratio in isolated anthers of rye genotypes differed in response to androgenesis induction. Mean values marked with the same letter do not differ significantly according to Duncan's multiple range test ($p<0.05$).

Fig. 4. GPX, GR, GST activity.

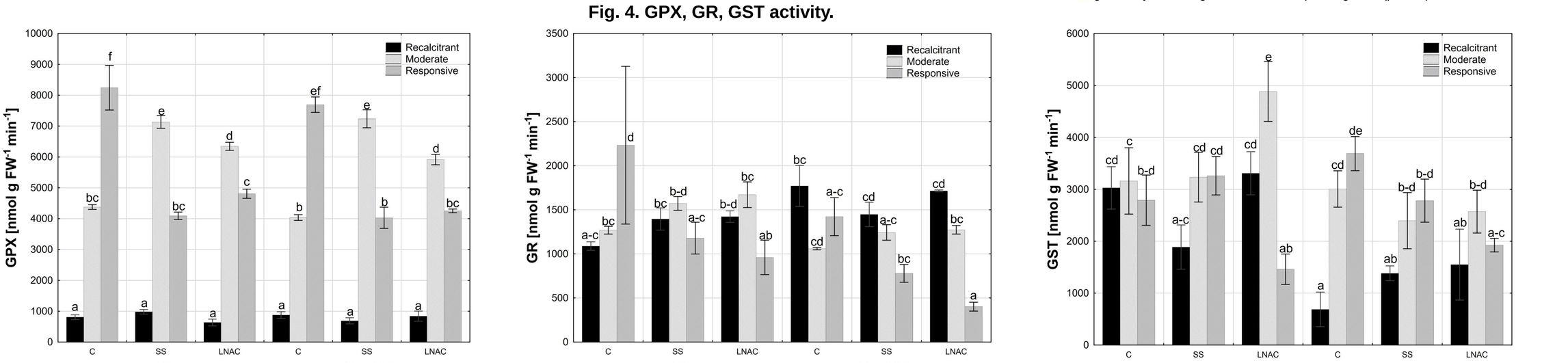

Fig. 4a **Fig. 4b** **Fig. 4c**

Fig. 4. Antioxidant enzymes activities GPX (a), GR (b) and GST (c) in anthers isolated from control/SS/LNAC-treated spikes of rye genotypes differed in response to androgenesis induced by LT or Mn_GSH. Mean values marked with the same letter do not differ significantly according to Duncan's multiple range test ($p<0.05$).

RESULTS

Rye genotypes differed significantly in response to androgenesis induction as well as in glutathione content catalysed by GPX, GR and GST enzymes (Figs. 3-4). Stress (LT, Mn), antioxidants (GSH, LNAC) and SS treatments affected the contents of endogenous GSH and oxidized glutathione (GSSG). The highest GSH/(GSH+GSSG) ratio was observed in control anthers of LT-treated recalcitrant and responsive lines and Mn_GSH-treated responsive lines. Interestingly, SS significantly reduced the GSH/(GSH+GSSG) ratio in both, recalcitrant (LT) and responsive (Mn_GSH) lines. The lowest GPX activity marked recalcitrant lines. SS and LNAC affected GPX activity in both, moderate and responsive lines (LT and Mn_GSH), while GR activity only in responsive lines (LT). GST demonstrated no significant response in activity compared with responsiveness to androgenesis induction.

CONCLUSIONS

- The GSH/(GSH+GSSG) ratio may be used as a marker of oxidative stress in isolated anthers of rye genotypes.
- Treatment with GSH, LNAC or SS affected the endogenous level of glutathione and probably enhanced the defence ability against the stress induced by LT spikes pre-treatment.

Literature

Zieliński K, Pociecha E, Krzewska M, Nowicka A, Fodor J, Żur I, Dubas E. 2015. 10th International Conference "Plant Functioning Under Environmental Stress", September 16-19, 2015, Krakow, Poland. Book of abstracts p.61.
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