The role of antioxidative system in the induction of microspore embryogenesis in anther culture of triticale (× *Triticosecale* Wittm.)

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Introduction

Stress applied to microspores, which *in planta* develop to pollen grains, can change the programme of its development and induce the process of microspore embryogenesis (ME). ME-derived doubled-haploid (DH) lines can be used as very unique models in basic studies, biotechnology and molecular engineering. Moreover, being totally homozygous, DH lines are widely incorporated into breeding programmes to accelerate production of new varieties.
The effectiveness of ME is under genomic control but to some extent can be modulated by the parameters of stress treatment. Various stresses can be used for ME initiation e.g. temperature shock, osmotic stress, starvation. As the generation of reactive oxygen species (ROS) is one of the first responses to stress, its involvement in microspore reprogramming has been postulated.
In triticale, cold treatment is used most often for microspore reprogramming. To further understand the processes triggered by ME-induction treatment, oxidative stress and antioxidative system activity were measured in the anthers of winter triticale (*xTriticosecale* Witm.) DH lines, which significantly differ in respect of their embryogenic potential.

Material and Methods

Five highly embryogenic (DH18, DH28, DH44, DH47, DH101) and five low responding (DH2, DH19, DH72, DH119, DH144) DH lines of winter triticale derived from F1 cross between German inbred line 'Saka 3006' and Polish cv. 'Modus' were in the study (Fig.1). The responsiveness of DH lines was estimated by anther culture method according to Wedzony (2003; Fig.2). In short, spikes were collected at the phase optimal for ME initiation, wrapped in plastic bags and aluminium foil and kept under low temperature (4°C), in the dark for 3 weeks. Aseptically excised anthers were placed in Petri dishes with modified C17 induction medium (Wang and Chen 1983). The medium was supplemented with 0.5 mg/l Kinetin, 1 mg/l Dicamba and 1 mg/l Picloram, 90 g/l maltose and 0.6 % agar; pH 5.8. The cultures were incubated in the dark at 28±1°C.
The effectiveness of ME induction was based on the number of androgenic structures produced per 100 anthers (AS/100A). Each dish containing 100 anthers collected from one spike was assumed to be a replicate.

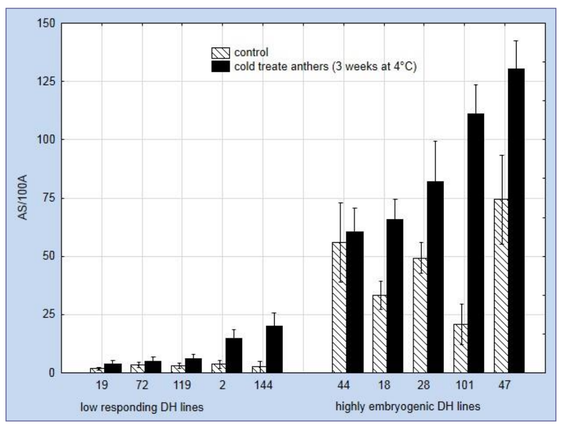

Fig.1. The efficiency of ME induction in DH lines of triticale with and without cold treatment (3 weeks at 4°). AS/100A – the number of androgenic structures produced per 100 anthers.

The oxidative stress connected with anther isolation and transfer to *in vitro* culture conditions, and cold-treatment was evaluated on the basis of the rate of superoxide radical ($O_2^{\cdot-}$) generation (nitroblue tetrazolium (NBT) method by Doke and Ohashi 1988; Fig.2) and hydrogen peroxide (H_2O_2) accumulation (Ishikawa et al. 1993). The activity of superoxide dismutase (SOD) isoenzymes was determined by standard gel electrophoresis (Laemmli 1970), whereas peroxidase (PEX) and catalase (CAT) activity was measured spectrophotometrically according to procedures described by Bergmeyer (1965) and Aebi (1984). The total activity of low molecular weight antioxidants (lmw Antiox) was analysed by DPPH (2,2-Diphenyl-1-picrylhydrazyl) method according to Wong et al. (2006). All analyses were performed on anthers isolated from freshly cut tillers (control) and from cold treated tillers (3 weeks at 4°C) in which microspores were induced towards embryogenesis.

Results and Conclusions

The performed analyses revealed that in all studied DH lines, the intensities of $O_2^{\cdot-}$ generation (not shown) and H_2O_2 accumulation (Fig.4) both in control and cold treated anthers were similar. It could be assumed that the stress related to anthers isolation predominated over the effects induced by the genotype and cold treatment.
Despite strong genomic control, the activity of all analysed elements of antioxidative system (SOD, CAT. PEX, lmw Antiox) was influenced by the treatment (ANOVA, p≤0,05). An interactive effect of genotype and stress treatment was also observed. The data for each DH line are presented in Figs 5-8. The summary showing the effect of embryogenic potential of studied DH lines is shown in Table 1.
All these results suggest that the activity of antioxidative system can be one of the factors that control the initiation of ME and influence it effectiveness. The mechanism of its action can be connected with the protection against excessive ROS accumulation and its deleterious effects on cell metabolism and structure.

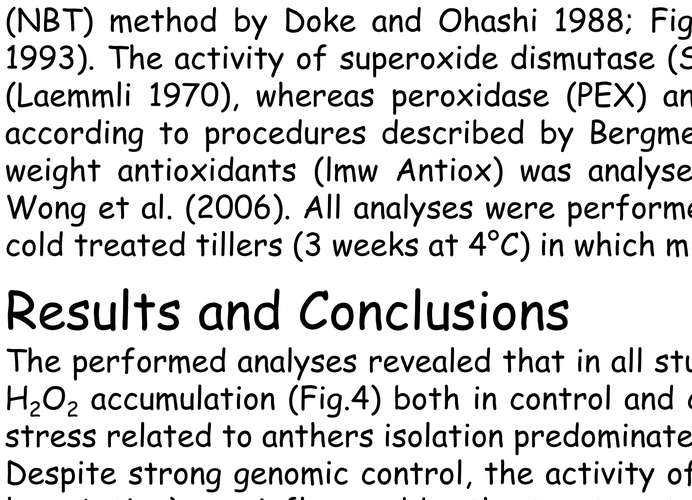

Fig.6. Activity of SOD isoforms in triticale anthers, isolated from freshly cut tillers (control; Fig.6A) and cold treated tillers (3 weeks at 4°C; Fig.6B).

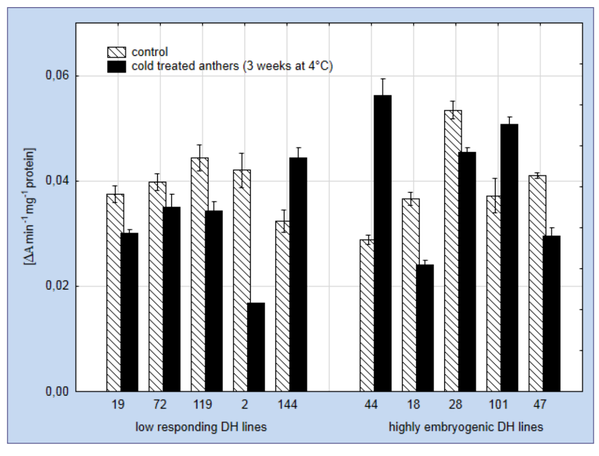

Fig.7. Activity of PEX in triticale anthers, isolated from freshly cut tillers (control) and cold-treated tillers (3 weeks at 4°C).

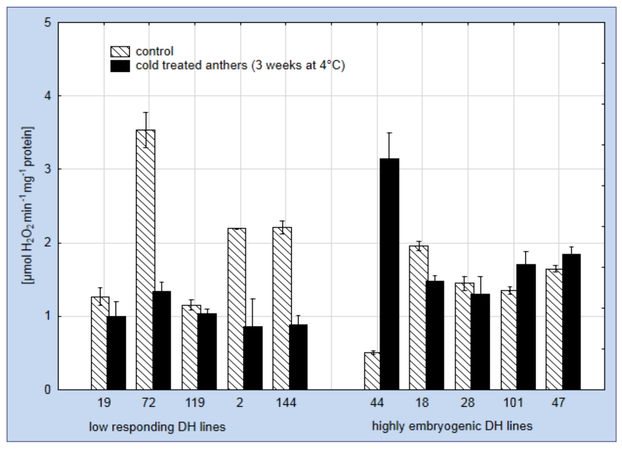

Fig.8. Activity of CAT in triticale anthers, isolated from freshly cut tillers (control) and cold-treated tillers (3 weeks at 4°C).

Parameter		Low responding DH lines		Highly embryogenic DH lines	
		Control	Cold treated	Control	Cold treated
Enzymatic antioxidants	CAT	2,0 ^{b}	1,0 ^{a}	1,4 ^{a}	1,9 ^{b}
	PEX	0,039 ^{b}	0,032 ^{a}	0,039 ^{ab}	0,04 ^{b}
Non-enzymatic antioxidants		31,3 ^{b}	33,8 ^{c}	28,6 ^{a}	31,5 ^{b}

Table 1. Parameters of antoxidative activity in anthers of DH lines of triticale characterized by various embryogenic potential. Activity measured in anthers isolated from freshly cut tillers (control) or cold treated tillers (3 weeks at 4°C).
CAT – catalase, PEX – peroxidase;
Data marked with the same letter do not differ significantly according to Duncan test (p≤0,05)

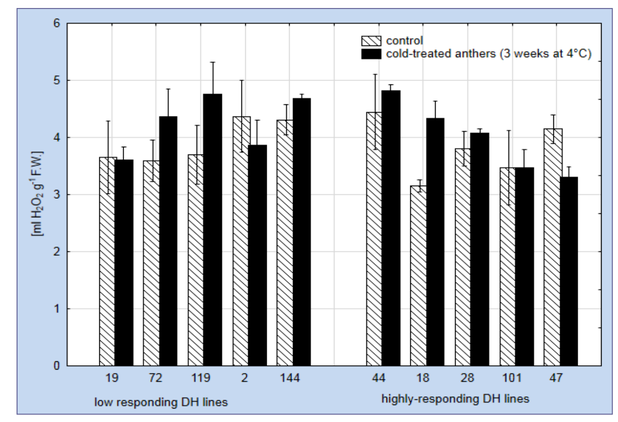

Fig.4. Accumulation of H_2O_2 in triticale anthers, isolated from freshly cut tillers (control) and cold treated tillers (3 weeks at 4°C).

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