Mapping of quantitative trait loci determining antioxidant activity in triticale (× *Triticosecale* Wittm.) anthers associated with androgenesis initiation

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Introduction

Androgenesis is an alternative, sporophytic pathway of development initiated in stress-treated cells of male gametophyte (microspores). The final product of this process – haploid (n) / doubled haploid (2n; DH) plants – are highly valued in many research areas and breeding practise. However, the effectiveness of androgenesis is highly genotype-depended, which limits its use for commercial purposes in many economically important crops. A considerable effort has been made to discover the mechanism underlying this process and the factors determining efficient DH production. Recent hypotheses propose reactive oxygen species (ROS) generation as signalling events and high antioxidant activity as an important prerequisite for effective androgenesis initiation.

In order to gain a better understanding of the mechanisms controlling triticale androgenesis, total water-soluble antioxidant activity was determined in triticale anthers in the phase of development optimal for androgenesis initiation and then after low-temperature (LT) stress treatment (3 weeks at 4°C) initiating microspore reprogramming. The analysis was conducted on a mapping population of hexaploid triticale, and the received data were then used to identify quantitative trait loci (QTL) responsible for controlling antioxidant capacity under both stress and non-stress conditions.

Material and Methods

A mapping population comprising 70 DH lines derived from F1 cross between German inbred line ’Saka 3006’ and Polish cv. ’Modus’ was used in the study. The responsiveness of DH lines was estimated by anther culture method according to Krzewska (2012; Fig. 1). In short, spikes were collected at the phase optimal for androgenesis initiation and kept under LT (4°C) in the dark for 3 weeks. Aseptically excised anthers were placed in Petri dishes with C17 induction medium supplemented with 0.5 mg l^{-1} Kinetin, 1 mg l^{-1} Dicamba and 1 mg l^{-1} Picloram, 90 g l^{-1} maltose and 0.6 % agar; pH 5.8. The cultures were incubated in the dark at 28±1°C. The effectiveness of induction was calculated on the basis of the number of embryo-like structures produced per 100 anthers (ELS/100A).

Total water-soluble antioxidant activity was determined by DPPH method according to Brand-Williams et al. (1995). QTL mapping was carried out by single marker analysis (SMA) and composite interval mapping (CIM) method using Windows QTL Cartographer version 2, and a recently constructed genetic map which consists of 1568 markers (155 SSRs, 28 AFLPs, 1385 DArTs) distributed within 21 linkage groups. Threshold LOD scores were calculated by 1000 permutations. The percentage of phenotypic variation was calculated with a single factor regression (R^2). The analyses were performed separately for two experimental replications.

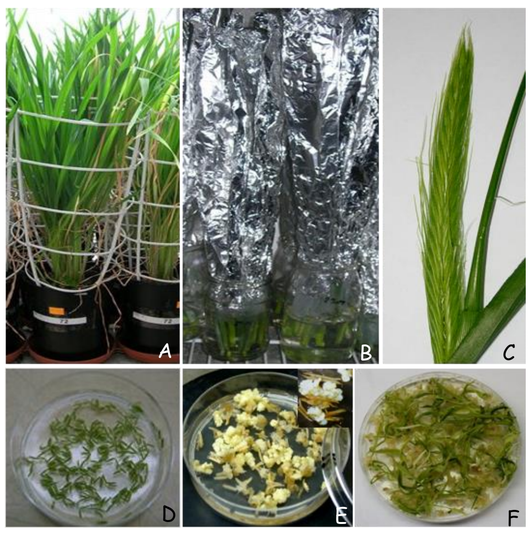

Fig.1. Anther culture of triticale. A) Donor plants; B) LT tillers treatment (3 weeks at 4°C); C) Spike at the developmental stage proper for ME initiation; D) Isolated anthers cultured on induction medium C17; E) ELS produced after 6-weeks *in vitro* culture; F) Plant regeneration.

Results

LT treatment distinctly enhanced antioxidative activity in the studied DH lines (Fig. 2), in a genetically-dependent range. Mean increase of antioxidative activity under LT for all studied DH lines amounted to 43% of the value measured in stress-free conditions. However, no significant association between this trait and androgenesis efficiency was detected.

CIM analysis localized three QTLs in anthers collected from control plants growing in stress-free conditions and five QTLs associated with antioxidative activity under LT stress (Fig. 3). All QTLs detected by CIM method were confirmed by SMA analysis ($p<0.05$) as carrying markers significantly associated with the studied traits. QTLs controlling antioxidative activity in control plants were localized on chromosome 3A and 4A (Table 1). Each of them explained about 20% of phenotypic variation. Another QTL was detected on chromosome 7R and was characterized by slightly lower R^2 (16%). Chromosomal regions controlling the response to LT stress were detected on 3A, 5R and 7R chromosomes with R^2 in 12.1-21.5% range. For almost all identified QTLs, the positive effect was inherited from the genotype ‘Saka 3006’. The only exception was locus *Qxfrsm-4A-1*, for which the positive effect originated from cv. ‘Modus’.

Fig.2. Total non-enzymatic antioxidant activity in anthers isolated from freshly cut tillers (control) and LT treated tillers (3 weeks at 4°C) of ‘Saka 3006’×‘Modus’ DH population of winter triticale.

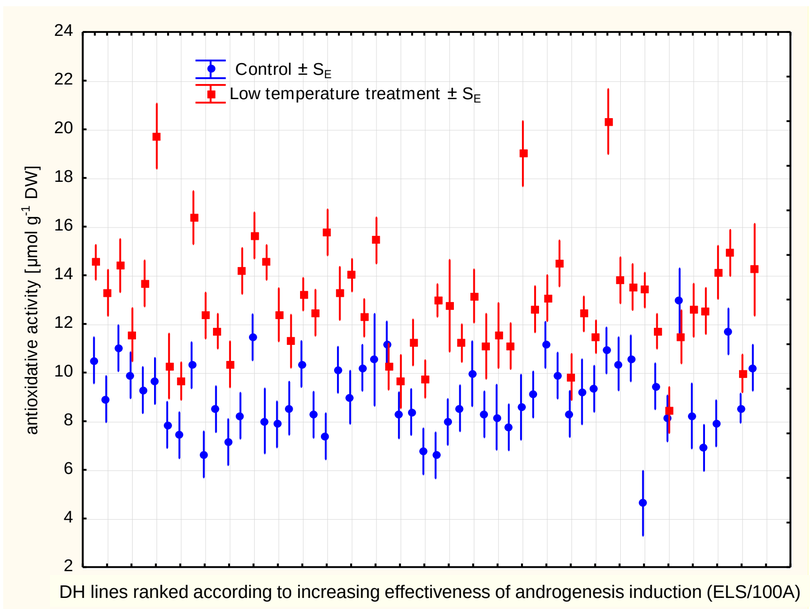

Table 1. Identification and molecular localization of loci of antioxidative activity in anthers isolated from freshly cut tillers (Ox/C) and after LT treatment (3 weeks at 4°C; Ox/LT) for winter triticale population ‘Saka 3006’×‘Modus’ by the use of CIM and SMA methods.

Trait	Loci QTL	QTL associated marker			LOD	R^2 (%)	Add
		name	position	*p* in SMA test			
Ox/C	Qxfrsm-3A-1	Xwmc532	48.6	*	4.2	20.18	1.039
	Qxfrsm-4A-1	wPt-1375	54.8	***	4.5	19.58	-0.786
	Qxfrsm-7R-1	rPt-399845	2	**	3.6	16.59	0.817
Ox/LT	Qxchsm-3A-2	wPt-9634	6.1	**	4.6	16.2	1.101
	Qxchsm-3A-3	rPt-401884	18.4	*	3.9	14.18	1.445
	Qxchsm-5R-1	rPt-506974	36.5	**	3.5	12.14	0.952
	Qxchsm-7R-2	rPt-7557	17.9	***	3.9	15.69	1.135
	Qxchsm-7R-3	wPt-4292	25.7	****	5.7	21.54	1.346

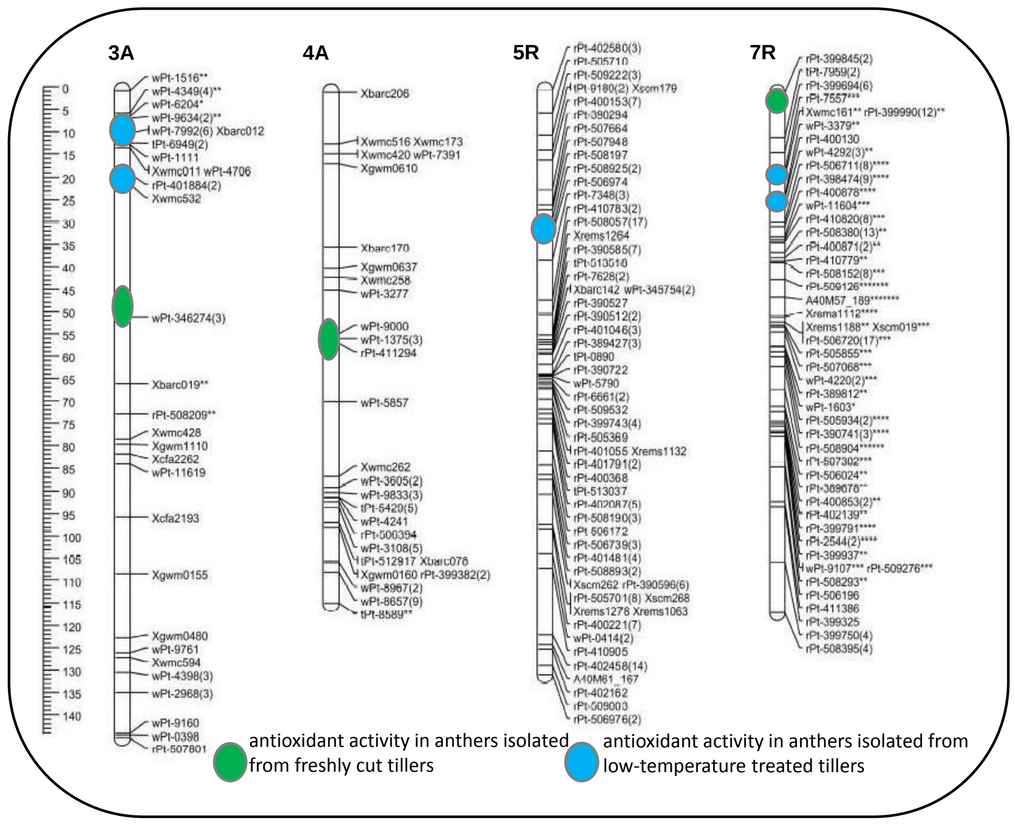

Fig. 3. Chromosome maps showing locations of QTLs associated with total non-enzymatic antioxidant activity in anthers of ‘Saka 3006’×‘Modus’ DH population of hexaploid winter triticale (x *Triticosecale* Wittm.)

Discussion and Conclusions

As the process of microspore reprogramming can be induced by various stress factors, the involvement of some non-specific defence reactions has been suggested. A good candidate for this role is accelerated generation of ROS. It disrupts redox homeostasis of cells, which can cause oxidative damage to various cell components. That is why ROS scavenging ability could be assumed as a crucial factor determining cell survival. On the other hand, ROS also act as signalling molecules involved in the control of numerous biological processes. The lack of direct linear correlation between the total activity of non-enzymatic antioxidants and the efficiency of androgenesis imply a complex character of the relations (possibly with a specific thresholds of positive effect). Other explanation is that a more important role is played by enzymatic components of the antioxidative system.

References

Krzewska et al. 2012. Quantitative trait loci associated with androgenic responsiveness in triticale (× *Triticosecale* Wittm.) anther culture. Plant Cell Reports 31:2099-2108

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