Modifications in the level of endogenous plant growth regulators in winter triticale (× *Triticosecale* Wittm.) anthers associated with the induction of microspore embryogenesis

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Introduction

The process of androgenesis is an example of plant cell totipotency phenomenon in which immature cells of the male gametophyte (microspores) switch towards embryogenic development. It has been proved that although the process of androgenesis is controlled by the plant's genome, it could be modified by factors influencing physiological state of the donor plant, among which the endogenous level of plant growth regulators (PGRs) is one of the most important. To analyse the effect of PGRs, endogenous levels of auxins (indolilo-3-acetic acid (IAA) and indolilo-butyrylic acid (IBA)), cytokinins (zeatin (Z), zeatin riboside (ZR) and kinetin (KIN)) and abscisic acid (ABA) were measured in the anthers of ten doubled haploid (DH) lines of hexaploid triticale (x*Triticosecale* Wittm.) highly different in respect of androgenic potential. PGRs concentration was analysed in anthers isolated from freshly cut tillers at the stage optimal for androgenesis induction and then after stress treatment (3 weeks at 4°C) which triggers microspore embryogenesis.

Material and Methods

Ten DH lines of triticale derived from F1 generation of a cross between inbred line 'Saka 3006' and cv. 'Modus' were used in the study. Donor plants were grown in a glasshouse at 25°C with a 16/8h (day/night) photoperiod. For androgenesis induction, the anther culture protocol described by Wedzony (2003) was used with minor modifications. The tillers were collected when the majority of microspores were at late-uninucleated stage of development and placed in darkness at 4°C for 3 weeks. Then the anthers form each spike were dissected and half of them were transferred onto C17 medium (Wang and Chen 1983) supplemented with 0,5 mg·dm^{-3} KIN, 1 mg·dm^{-3} Dicamba, 1 mg·dm^{-3} Picloram, 90 g·dm^{-3} maltose and 0,6 % agar (pH 5,8). The cultures were kept in darkness at 28±1°C. After 6-week culture the number of androgenic structures (AS) produced per 100 anthers was calculated (AS/100A). One Petri dish (Ø 6cm) containing 100 anthers was considered as a replication.

The second half of the anthers were immediately frozen in liquid N_2 and used for PGRs analyses. The auxins and cytokinins analyses were performed by the HPLC method according to Dobrev and Kamínek (2002) and modified by Stefancic et al. (2007), whereas ABA was measured by ELISA (Walker-Simmons and Abrams 1991).

Both in anther culture and PGRs analyses, the anthers dissected from freshly cut tillers were used as the control.

Results and Conclusions

The obtained results confirmed earlier estimation of androgenic potential of all DH lines and significant variation among selected genotypes, which could be classified into one of two groups: 'low responsive' (LR) or 'highly responsive' (HR) to androgenesis induction treatment (Fig.1-2). The mean androgenesis efficiency for anthers isolated from freshly cut tillers amounted to 3 and 45 AS/100A for LR and HR genotypes, respectively. For both groups, low temperature treatment significantly increased the effectiveness of the process to 10 (LR) and 89 (HR) AS/100A.

The endogenous level of all analysed PGRs in anthers of studied DH lines was significantly depended on the plant genotype and tillers treatment. Low temperature treatment significantly increased IAA, IBA and ABA levels (Fig.3a,c) and differentially changed the content of various isomers of Z and ZR (Fig.3b). Generally, in triticale anthers *cis*-isomers of Z and ZR predominated. In response to low temperature, the amount of *trans*-isomers decreased and instead *cis*-isomers were accumulated. High amount of KIN was also detected in the majority of studied DH lines of triticale (Fig.3b) and significant decrease of its content under low temperature treatment was observed.

It was also revealed that HR triticale lines were characterized by significantly lower IAA and higher IBA content in comparison with LR ones (Table 1). Moreover, in non-stressed anthers the level of *trans*-isomers of Z and ZR was higher in HR genotypes in comparison with LR ones. The opposite relation was observed in the case of *cis*-Z isomer. After low temperature treatment *trans*-Z, *cis*-Z and *cis*-ZR content was higher in HR genotypes. In response to stress treatment, HR genotypes also accumulated a significantly higher amount of ABA than LR ones.

Generally, the auxin/cytokinin ratio for LR genotypes (28 and 25 for control and low temperature treated anthers, respectively) was higher in comparison with HR genotypes (25 and 18), especially in the anthers after application of androgenesis-inducing treatment. It seems that such variation in endogenous PGRs balance could be at least one of the reasons for different androgenesis responsiveness.

Table 1. The mean content of PGRs in anthers of ten DH lines of triticale classified as 'low' or 'highly responsive' to androgenesis induction treatment (3 weeks at 4°C). PGRs content was measured in anthers isolated from freshly cut tillers (control; C) and low-temperature treated tillers (LT). Red colour means significant difference between groups (p≤ 0,05).

Androgenic potential	**Auxins** [µg g^{-1} DW]				**Cytokinins** [ng g^{-1} DW]										**ABA** [ng g^{-1} DW]	
	IAA		IBA		*trans*-Z		*cis*-Z		*trans*-ZR		*cis*-ZR		Kinetin			
	C	LT	C	LT	C	LT	C	LT	C	LT	C	LT	C	LT	C	LT
Low responsive	3,8	4,8	0,04	0,06	1,5	0,8	44,5	70,4	1,5	1,5	83,3	112,9	6,9	6,4	230,2	511,2
Highly responsive	2,9	3,7	0,05	0,08	2,1	1,1	36,1	78,4	2,0	1,4	73,2	126,5	6,0	4,5	213,1	835,1

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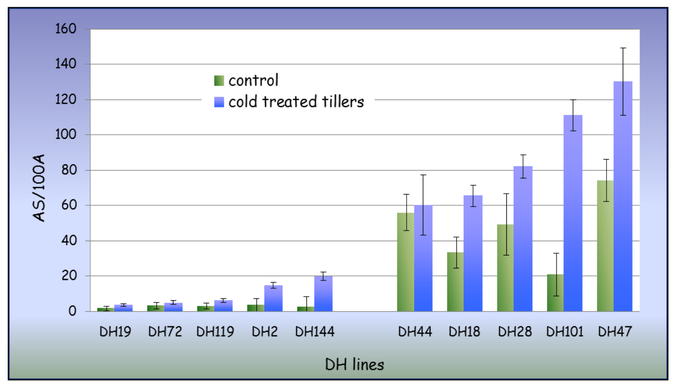
Fig 1. The effect of genotype and low temperature treatment on androgenesis efficiency in anther culture of triticale DH lines 'Saka 3006'x 'Modus'. The data are the means of 15 replication (plates containing 100 anthers) ± Sd. AS/100A – the number of androgenic structures per 100 isolated anthers.



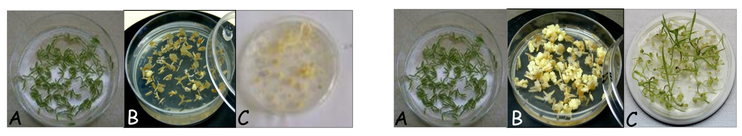
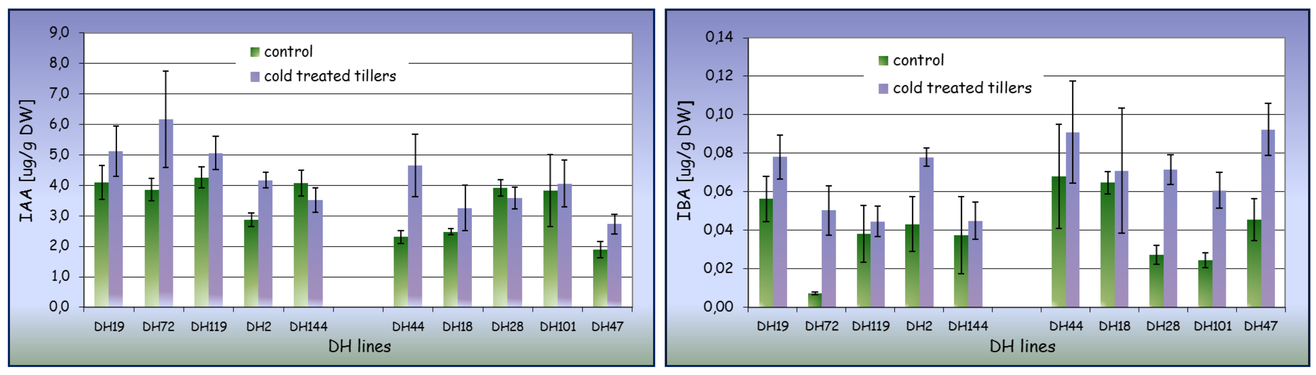


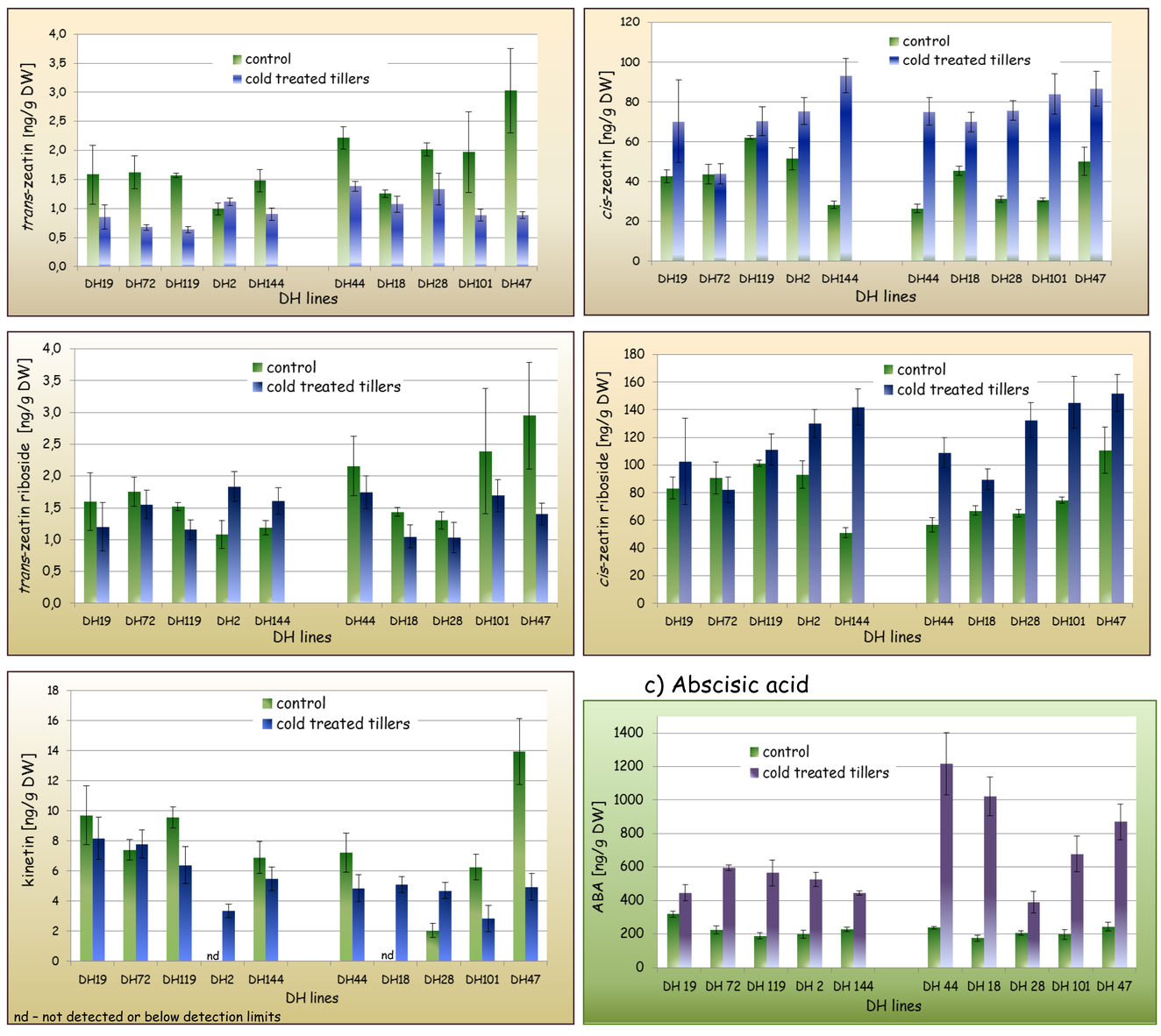
Fig.2. Selected examples of various androgenesis responsiveness in anther culture of studied DH lines of triticale 'Saka 3006'x'Modus' **A)** Anthers on induction medium C17 just after isolation; **B)** Androgenic structures after 6-week culture on C17 medium; **C)** Regeneration on mod. 190-2 medium (after 10th week of culture).

Fig.3. The effect of genotype and androgenesis-induction treatment on PGRs concentration in triticale anthers. The data are the means of 5-6 replications ±Sd.

a) Auxins



b) Cytokinins



c) Abscisic acid

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