Identifying QTLs associated with albino plant formation and some new facts concerning green versus albino ratio determinants in triticale (×Triticecale Wittm.) anther culture

Monika Krzewska · Ilona Czyczyło-Mysza · Ewa Dubas · Gabriela Gołąbiowska-Pikania · Iwona Żur

Received: 26 February 2015 / Accepted: 30 July 2015 / Published online: 15 July 2015
© The Author(s) 2015. This article is published with open access at Springerlink.com

Abstract High frequency of albino haploids/doubled haploids (DHs), regenerated in androgenic cultures is one of the major obstacles that limit incorporation of DHs technology into cereal breeding programs. Therefore, quantitative trait loci (QTL) associated with albino plant production in triticale anther cultures were analyzed using the population of 90 DH lines derived from F1 cross ‘Saka 3006’ × ‘Modus’. Composite interval mapping (CIM) and single marker analysis (SMA) in Windows QTL Cartographer ver 2.5 were used to localize the major QTLs. CIM method revealed seven QTLs with LOD scores between 2.9 and 5.6 on five chromosomes from B to R subgenomes (3B, 4B, 4R, 5R and 7R). Effects of all QTLs explained 8.3–17.6 % of the phenotypic variation and were confirmed by SMA analysis. Additionally SMA revealed another seven markers on chromosomes: 2AL, 2BL, 3B, 2BS, 6AL, 2RS, 3R and 4R associated with QTL for albino plant regeneration (p < 0.01). The additional experiment with ten DH lines varied significantly in their androgenic responsiveness was conducted to analyze the changes in the level of oxidative stress, antioxidative system activity and endogenous hormonal balance associated with androgenesis-inducing low temperature stress treatment (3 weeks at 4 °C). The correlation analysis between albino/green plant regeneration ability and analyzed traits were performed by using Spearman Rank test (p ≤ 0.05). Revealed associations may suggest that some level of oxidative stress is necessary for transition from a non-photosynthetic proplastids to the functional chloroplasts. On the other hand, the efficient antioxidative enzyme system and endogenous hormonal balance are also very important.

Key message Fourteen chromosome regions were indicated to control albino plant formation during triticale anther culture. Additionally, reactive oxygen species (ROS) generation, antioxidative system activity and hormonal balance were discussed as determinants in androgenesis.

Keywords Anther culture · Albino plants · Composite interval mapping · QTL · Winter triticale

Introduction

Microspore embryogenesis (ME), also called androgenesis, is defined as the reproduction of an individual with genetically exclusive male origin (Segui-Simarro 2010). This remarkable process is considered as one of
the fastest and simplest route to obtain haploid and doubled haploid plants (DHs), which are very good tools for breeding programs, as well as for biotechnology and molecular studies. However, the androgenic effectiveness is expressed only under certain circumstances as a consequence of an environmental stress (Bonet et al. 1998) and it is controlled by an interaction of genetic and physiological factors.

One of very important problems in androgenic cultures of monocots is the formation of chlorophyll-deficient, so called ‘albino’ or ‘albinotic’ plants. This phenomenon is observed in majority of cereals like wheat (Andersen et al. 1987), barley (Knudsen et al. 1989), rice (Guiderdoni et al. 1992), rye (Immonen 1999) and oat (Kiviharju and Pehu 1998) with the frequency that may vary from 5 to 100 % of regenerants. Also in triticale, high frequencies of chlorophyll-defective regenerants have been usually reported (González et al. 1997; Ponitka et al. 1999; Schumann 1990) sometimes prevailing the frequency of properly developed plants (Pauk et al. 2000). Albinotic plants cannot survive in natural environment, outside in vitro culture, and have no agronomic value. Despite many efforts that have been made to bring the better understanding of the mechanisms leading to albino plant formation, the primary reasons are still unknown.

In order to elucidate the mechanism of albino plant formation researchers have focused on three areas: cytology, plastid genomics and the nuclear genome, identifying numerous factors as involved in control of the process (Calić et al. 2013; Immonen and Robinson 2000; Jacquard et al. 2009; Lantos et al. 2013; Slusarkiewicz-Jarzina and Ponitka 1997; Wojnarowiez et al. 2002). Some researchers claim that origin of albinism in some cultivars is determined at the earliest phases of ME and obtaining green regenerants depends on the state of microspore plastids at the moment of sampling (Caredda et al. 2004, 2000; Muñoz-Amatriain et al. 2009).

Molecular examinations on barley (Muñoz-Amatriain et al. 2009) revealed that genes related to stress response, transcription and translation regulation, and degradation of pollen-specific proteins were associated with green plant production, while expression of genes related to plastid development was associated with albino plant regeneration. Albino plants showed an aberrant form of plastids and, frequently, deletions in their plastid DNA (Harada et al. 1991; Hofinger et al. 2000). It was proved that transcript levels of plastid encoded genes for photosynthetic proteins and ribosomal RNA were generally heavily reduced in albino plants in relation to green plants (Ankele et al. 2005; Dunford and Walden 1991; Hofinger et al. 2000). Translation deficiencies and the modified transcript pattern in androgenic albino plants could be explained by lack of functional plastid ribosomes (Hofinger et al. 2000). Genetic studies support the hypothesis that nuclear factors contribute to the formation of albino plants (Ankele et al. 2005; Zhou and Konzak 1992). As most of the proteins of photosynthesis-related complexes are encoded in the nuclear genome the participation of nuclear genes in this phenomenon seems to be dominant. Muñoz-Amatriain et al. (2009) found that high number of chlorophyll-deficient plants in barley was associated with the expression of three genes that could be related to plastid development. One of them was gene with homology to DAG, which is essential for chloroplast development from proplastids, and acts very early in chloroplast development (Chatterjee et al. 1996). The second gene encoded a class B ankyrin repeat protein, which is involved in plastid differentiation (Garcion et al. 2006). The third one was a transcription factor, that encodes abscisic acid-insensitive 3 (ABI3), which is important for plastid identity and could influence on plastid ultrastructure (Rohde et al. 2000).

Statistical approach with the use of quantitative trait loci (QTL) analysis provide a new possibility for the identification of the molecular control over albinism. So far, QTLs for green plant percentage were mapped in wheat (Torp and Andersen 2009; Torp et al. 2001), rye (Grosse et al. 1996) barley (Muñoz-Amatriain et al. 2008) and triticale (González et al. 2005; Krzewska et al. 2012) and many different genes affecting the trait have been recognized. However, little is known about the molecular and physiological mechanisms leading to the formation of albino plants and further studies are necessary to help in understanding this phenomenon.

In this study, a population of 90 DH lines derived from the cross between hexaploid winter triticale (*Triticosecale* Wittm.) ‘Saka 3006’ and ‘Modus’
was used for identification of QTLs associated with albino plant production by anther culture method. The same mapping population together with produced for it well-saturated genetic linkage map (Tyrka et al. 2011) was successfully used in several previous studies (Krzewska et al. 2012; Szychska-Hebda et al. 2011; Żur et al. 2012). Among others, the QTLs associated with androgenic structure formation and green plant regeneration has been identified (Krzewska et al. 2012). In this report, the results of QTL analysis showing the association with albino plant formation supplement earlier published reports. Moreover, new statistical approach allows for acquisition of data showing possible associations between ROS generation, antioxidative system activity, endogenous hormones level and the effectiveness of androgenesis. These data has been received from additional experiments in which the anthers of highly responsive and highly recalcitrant DH lines selected out from ‘Saka 3006’ ‘Modus’ mapping population and identified as highly responsive (1–5) and highly recalcitrant (6–10) were used in the additional experiments. In all experiments, the seeds were germinated in the dark for 2 days at room temperature, then placed in perlite pre-soaked with Hoagland’s salt solution and vernalized (7 weeks at 4 °C with a photoperiod of 8/16 h day/night). Vernalized seedlings were planted in a mixture of soil, deacidified substrate peat and sand (2/2/1; v/v/v) and grown in a greenhouse at 20 ± 2 °C with 16/8 h (day/night) photoperiod until the flowering.

Materials and methods

Plant material and growth conditions

The mapping population of 90 DH lines derived from F1 generation of a cross between winter triticale inbred line ‘Saka 3006’ and cv. ‘Modus’ by crosses with maize method together with both DH parental lines were used in this study. The population was created by Dr Eva Bauer from the State Plant Breeding Institute, Hohenheim University in Stuttgart, Germany and kindly provided for this research. Several DH lines, namely: DH18, DH28, DH44, DH47, DH101 (1–5) and DH2, DH19, DH72, DH119, DH144 (6–10) selected out from ‘Saka 3006’ × ‘Modus’ mapping population and identified as highly responsive (1–5) and highly recalcitrant (6–10) were used in the additional experiments. In all experiments, the seeds were germinated in the dark for 2 days at room temperature.
calculated as the mean from at least ten replications, with a 60 x 15 mm Petri dishes containing 100 anthers from one spike considered as one replication.

Statistical and QTL analysis

All statistical analyses were performed using STATISTICA version 10.0 (Stat Soft Inc., USA, 2011) package. For each tested variable the normal distribution of scores has been verified by Shapiro–Wilk test to validate the use of the parametric tests. The effect of tested variables was examined by multi-factor analysis of variance (ANOVA). Variables with non-normal distributed data were analyzed with non-parametric Kruskal–Wallis tests. Non-parametric Spearman’s Rank-Order Correlation coefficients (R) were used to analyze the association between variables with non-normal distribution of scores.

Genetic map for used in the study triticale population was constructed by Tyrka et al. (2011) and consists of 1568 markers (155 SSRs, 28 AFLPs, 1385 DArTs) distributed within 21 linkage groups. The map covers 2397 cM and the average distance between markers is 4.1 cM.

QTL analysis was performed with Windows QTL Cartographer version 2.5 (Wang et al. 2012) by using two methods: single marker analysis (SMA) and composite interval mapping (CIM). A LOD threshold of 3.0 was used to detect QTL; however, in the case of consistent detections a LOD score beyond 2.0 was considered significant. The percentage of phenotypic variation was calculated with a single factor regression (R²).

Table 1 Effect of tested variables on androgenic effectiveness in anthers culture of 92 DH lines of winter triticale mapping population ‘Saka 3006’ × ‘Modus’

| Sources of variation | SS       | df | MS    | F    | P      |
|----------------------|----------|----|-------|------|--------|
| Green plants regeneration (GR/100AS) |          |    |       |      |        |
| (1) Genotype         | 19,220   | 91 | 211   | 3.29 | ***    |
| (2) Experiment replication | 3804 | 2  | 1902  | 29.66| ***    |
| (1) * (2)            | 13,727   | 182| 75    | 1.18 | NS     |
| Albino plants regeneration (AR/100AS) |          |    |       |      |        |
| (1) Genotype         | 12,300   | 91 | 135.7 | 2.83 | ***    |
| (2) Experiment replication | 1715 | 2  | 857.5 | 17.9 | ***    |
| (1) * (2)            | 8367     | 182| 46    | 0.96 | NS     |

SS sum of squares, df degrees of freedom, MS mean square, F statistic in ANOVA, p probability, NS not statistically significant

*** p < 0.001

Table 2 Mean efficiency of regeneration in anther cultures of parental DH lines (DH ‘Saka 3006’, DH ‘Modus’) and derived from their F1 cross hybrid population of 90 DH lines. Offspring DH population is characterized also by the extremes range (min–max)

|                  | GR/100AS | AR/100AS | GR/AR (AS) | GR/100A | AR/100A | GR/AR (A) |
|------------------|----------|----------|------------|---------|---------|-----------|
| DH ‘Saka 3006’   | 4.4 ± 1.5 | 6.2 ± 1.6 | 0.7        | 1.6 ± 0.5 | 1.9 ± 0.4 | 0.8       |
| DH ‘Modus’       | 7.0 ± 2.1 | 7.4 ± 1.1 | 0.9        | 6.7 ± 1.5 | 8.1 ± 1.8 | 0.8       |
| Mean for DHs progeny | 7.9 ± 0.2 | 7.6 ± 0.2 | 1.0        | 5.0 ± 0.2 | 4.4 ± 0.1 | 1.1       |
| Max–min range for DHs progeny | 0–31 | 0–31 | 0–10 | 0–38 | 0–19 | 0–11 |

The data are the mean ± SD of three separate experimental replications with 10 biological replications (plates containing 100 anthers) each. GR/100AS, number of green regenerants (GR) per 100 androgenic structures (AS); AR/100AS, the number of albino regenerants (AR) per 100 androgenic structures (AS); GR/100A, number of green regenerants (GR) per 100 anthers (A); AR/100A, the number of albino regenerants (AR) per 100 anthers (A); GR/AR (AS), the ratio between GR and AR frequency calculated per 100AS; GR/AR (A), the ratio between GR and AR frequency calculated per 100 A
Results

Green/albino plant production in triticale anther cultures

In all experimental replicates of the main study circa 53% of regenerated plants were albinotic. Variation analysis (Table 1) showed significant influence (p ≤ 0.001) of both donor plant genotype and experimental replication on regeneration effectiveness. The effect of interaction between variables was not significant. To characterize precisely the regeneration ability of produced androgenic structures, six parameters were recorded and analyzed (Table 2). Received data indicate that parental lines were similar in respect of albino regenerants frequency (AR/100AS) whereas the number of regenerated green plants (GR/100AS) was almost 2-fold higher in the case of DH line ‘Modus’ (Table 2). The significant differences were also found in parameters characterizing final androgenic efficiency (GR/100A, AR/100A), where both green and albino plant production were more than 4-fold higher for paternal genotype ‘Modus’. However, very similar GR/AR ratios suggest that this difference is the result of variation in the number of produced AS and their total regeneration ability. In the studied DH progeny a much wider variation with extremes significantly exceeding characteristics of parental genotypes was observed (Table 2). Nevertheless, mean effectiveness of green and albino plant formation was very similar.

The results of plant regeneration in anther cultures of ten selected DH lines significantly different in their androgenic potential are presented on Fig. 1. It could be seen that both highly recalcitrant and highly responsive DH lines of triticale were characterized by rather low regeneration ability as only up to 9 and 6% of AR/100AS the number of albino regenerated plants per 100 androgenic structures, GR/100AS the number of green regenerants per 100 androgenic structures, AR/100A the number of albino regenerated plants per 100 anthers, GR/100A the number of green regenerants per 100 anthers, FC freshly cut tillers, LT cold treated tillers (3 weeks at 4 °C)
The CIM analysis revealed seven QTLs localized on five chromosomes from B and R subgenomes associated with albino plants regeneration (Table 4; Fig. 2). All of them were confirmed by SMA analysis as regions containing the markers significantly linked to the studied trait. Additionally, the results of SMA analysis indicated seven statistically significant \( p < 0.01 \) markers on chromosomes 2AL.2BL, 3B, 2BS.6AL, 2RS.3R and 4R (Table 5).

The most significant QTLs associated with AR/100AS were located on chromosome 5R with high LOD scores from 3.1 to 5.6. The highest percentage of phenotypic variance, almost 17 %, was explained by the locus \( QARS_{sm}-5R-1 \). All of these QTLs show a positive effect when the allele comes from cv. ‘Modus’.

### Table 3

| Trait | QTL | Flanking markers (cM) | LOD | \( R^2 \) (%) | Add | Marker linked to a QTL |
|-------|-----|-----------------------|-----|---------------|-----|-------------------------|
| AR/100AS | \( QARS_{sm}-3B-1 \) | wPt-1191 (26)–tPt-5771 (28) | 2.9 | 8.28 | 1.24 | tPt-5771 ** |
|      | \( QARS_{sm}-5R-1 \) | tPt-7245 (50)–Xrems1264 (54) | 5.6 | 16.85 | -1.75 | tPt-508057 **** |
|      | \( QARS_{sm}-5R-2 \) | Xbarc142 (58)–rPt-509048 (59) | 3.1 | 11.22 | -1.46 | wPt-345754 **** |
|      | \( QARS_{sm}-5R-3 \) | rPt-509532 (65)–rPt-505369 (66) | 4.3 | 12.98 | -1.57 | rPt-399743 **** |
| AR/100A | \( QARsm-4B \) | Xgwm0251 (51)–Xgwm0251 (53) | 4.8 | 17.56 | 1.16 | Xgwm0251 ** |
|      | \( QARsm-4R-1 \) | wPt-6160 (125)–wPt-11641 (127) | 3.5 | 11.76 | -1.14 | wPt-11641 *** |
|      | \( QARsm-7R \) | rPt-411386 (91)–rPt-399325 (92) | 5.6 | 17.34 | -1.44 | rPt-399325 *** |

AR/100AS, the number of albino regenerated plants per 100 androgenic structures; AR/100A, the number of albino regenerated plants per 100 anthers; LOD, logarithm of the odds for peaked marker; \( R^2 \) (%), % of phenotypic variance explained by the QTL; Add, additive effect of the ‘Saka 3006’ allele; p, significance in SMA analysis **, ***, **** p < 0.01, 0.001, 0.0001, respectively

\( a \) cM position of the marker on the genetic map of a given chromosome

\( b \) The closest marker to LOD peak
(QARSsm-3B-1) was 2.9 and peaked at DArT marker tPt-5771. Moreover, localized close to described QTL connected with AR/100AS. Although, the most significant marker (p < 0.001) was found in the telomeric region of the short arm of the chromosome 2BS.6AL (Table 5; Fig. 2).

The major QTLs associated with the second studied variable AR/100A were localized on four chromosomes: 4B, 2RS.3R, 4R and 7R (Fig. 2). It seems that one of the most important genomic region for this parameter is interval between rPt-411386 (91 cM) and rPt-399325 (92 cM) markers on chromosome 7R. This QTL explained over 17% of phenotypic variation with values of LOD 5.6. The most QTLs were detected on chromosome 4R. One of them was revealed by CIM method and other three markers were found in SMA analysis (Tables 4, 5). Moreover, for all these QTLs the positive effect was inherited from ‘Modus’. The next QTL, which peaked at SSR marker Xgwm0251, was detected on chromosome 4B and only in this case the additive effect comes from ‘Saka 3006’ allele.

### Table 5 Additional significant markers associated with studied traits revealed in single marker analysis

| Trait     | QTL         | Marker (cM) | p    |
|-----------|-------------|-------------|------|
| AR/100AS  | QARSsm-2AL.2BL | rPt-508871 (56) | **   |
|           | QARSsm-3B-2   | Xgwm0285 (37) | **   |
|           | QARSsm-2BS.6AL | tPt-4602 (2) | ***  |
| AR/100A   | QARsm-2RS.3R  | rPt-399503 (76) | **   |
|           | QARsm-4R-2    | rPt-411069 (115) | **** |
|           | QARsm-4R-3    | rPt-401399 (119) | **** |
|           | QARsm-4R-4    | Xrems1024 (138) | ***  |

AR/100AS, the number of albino regenerated plants per 100 androgenic structures; AR/100A, the number of albino regenerated plants per 100 anthers; p, significance in SMA analysis **, ***, **** p < 0.01, 0.001, 0.0001, respectively

Correlation analysis was performed with the use of Spearman Rank test (p ≤ 0.05) for the data received in additional experiments with ten DH line of triticale used as the object of the study.

Presented here (Table 6), are the results concerning the associations between parameters of plant regeneration effectiveness (GR/100AS, AR/100AS, GR/AR (AS)), final androgenesis effectiveness (GR/100A, AR/100A, GR/AR (A)), endogenous level of plant growth regulators (IAA, IBA, trans and cis isomers of zeatin (tZ, cZ) and zeatin riboside (tZR, cZR), kinetin (Kin) and abscisic acid (ABA), generation of ROS (superoxide anion (O−), hydrogen peroxide (H2O2)) as well as the activity of antioxidative enzymes (superoxide dismutase (SOD), catalase (CAT), peroxidase (POX)) and total activity of low molecular weight antioxidants (AntiOx).

Due to the fact that recalcitrant DH lines were much more varied in respect of regeneration ability in comparison with responsive DH lines, higher number of significant associations has been found when the statistical analysis was done separately for each group of DH lines.

Correlation analysis for responsive DH lines in the control conditions (FC, Table 6) detected significant associations between GR/AR (AS) and CAT activity, AR/100A and AntiOx, and GR/AR (A) and H2O2 generation suggesting negative effect of oxidative stress and positive influence of more effective antioxidative system. On the contrary, in the group of recalcitrant DH lines, higher activity of POX was negatively correlated with green plant regeneration (GR/100AS) and final green plant production (GR/100A) and simultaneously positively correlated with albino plant regeneration/final effectiveness (AR/100AS, AR/100A). Significant correlations were found also between cZ, cZR and both green and albino plant regeneration/final production (GR/100AS, AR/100AS, GR/100A, AR/100A). The level of cis isomers of Z and ZR correlated negatively with green plants regeneration/final effectiveness (GR/100AS, GR/100A) and positively with albino plant regeneration/final effectiveness (AR/100AS, AR/100A). Negative correlation was found also between ABA level and albino plant regeneration/final effectiveness (AR/100AS, AR/100A).

For responsive DH lines after LT tillers pre-treatment, the effectiveness of green plant regeneration (GR/100AS) was positively correlated with AntiOx and negatively correlated with ABA concentration. Albino plant regeneration/final effectiveness (AR/100AS, AR/100A) looks to be negatively influenced by higher level of IAA whereas GR/AR (AS) negatively correlated with the activity of SOD. Regeneration effectiveness of recalcitrant genotypes could be determined mainly by hormonal background. Green plant regeneration (GR/100AS) was positively...
correlated with \( cZ \) and \( cZR \) levels and negatively correlated with IAA. Higher concentration of Kin seems to diminish both green and albino plant regeneration (GR/100AS, AR/100AS). The content of IBA correlated negatively with GR/AR (AS) whereas \( tZR \) positively correlated with AR/100AS. Quite unexpectedly, the final effectiveness of albino plant production (AR/100A) was correlated with both hormonal balance and the capacity of antioxidative system. Additionally to the parameters that seems to be significantly associated with albino plant regeneration (\( tZ \), Kin), positive correlation with \( tZ \) and negative associations with SOD, CAT and AntiOx activities were also detected.

### Table 6

Spearman Rank Correlation test (\( p \leq 0.05 \)) for the data received with ten DH line of triticale significantly different in androgenic responsiveness

| Parameter | Endogenous hormone levels | Oxidative stress | Antioxidative activity |
|-----------|---------------------------|------------------|-----------------------|
|           | ABA | IAA | IBA | tZ | cZ | tZR | cZR | Kin | O\(^{-}\) | \( H_2O_2 \) | SOD | CAT | POX | AntiOx |
| **Responsive DH lines—control** | | | | | | | | | | | | | | |
| GR/100AS | | | | | | | | | | | | | | |
| AR/100AS | | | | | | | | | | | | | | |
| GR/AR (AS) | | | | | | | | | | | | | | 0.90 |
| GR/100A | | | | | | | | | | | | | | -0.90 |
| AR/100A | | | | | | | | | | | | | | -0.90 |
| **Recalcitrant DH lines—control** | | | | | | | | | | | | | | |
| GR/100AS | | | | | | | | | | | | | | -0.97 |
| AR/100AS | -0.89 | | | 0.89 | 0.89 | | | | | | | | 0.89 |
| GR/AR (AS) | | | | | | | | | | | | | | |
| GR/100A | | | | | | | | | | | | | | -0.97 |
| AR/100A | -0.89 | | | 0.89 | 0.89 | | | | | | | | 0.89 |
| **Responsive DH lines—cold treated** | | | | | | | | | | | | | | |
| GR/100AS | | | | | | | | | | | | | | -0.90 |
| AR/100AS | | | | | | | | | | | | | | 0.90 |
| GR/AR (AS) | | | | | | | | | | | | | | -0.90 |
| GR/100A | | | | | | | | | | | | | | -0.90 |
| AR/100A | | | | | | | | | | | | | | |
| **Recalcitrant DH lines—cold treated** | | | | | | | | | | | | | | |
| GR/100AS | | | | | | | | | | | | | | -0.90 |
| AR/100AS | | | | | | | | | | | | | | 0.90 |
| GR/AR (AS) | | | | | | | | | | | | | | -0.95 |
| GR/100A | | | | | | | | | | | | | | 0.90 |
| AR/100A | -0.90 | | | 0.90 | 0.90 | | | | | | | | 0.90 |
| GR/AR (A) | | | | | | | | | | | | | | -0.95 |

GR/100AS, the number of green regenerants per 100 androgenic structures; AR/100AS, the number of albino regenerants per 100 androgenic structures; GR/AR (AS), the ratio between GR and AR frequency calculated per 100 AS; GR/100A, the number of green regenerants per 100 anthers; AR/100A, the number of albino regenerants per 100 anthers; GR/AR (A), the ratio between GR and AR frequency calculated per 100 A; ABA abscisic acid; IAA indole-3-acetic-acid; IBA indole-3-butyric acid; \( tZ \) trans zeatin; \( cZ \) cis zeatin; \( tZR \) trans zeatinriboside; \( cZR \) cis zeatinriboside; Kin kinetin; \( O^{-}\), superoxide anion; \( H_2O_2 \), hydrogen peroxide; SOD superoxide dismutase; CAT catalase; POX peroxidise; AntiOx low molecular weight antioxidants.

---

Euphytica (2015) 206:263–278 271
Generally, no correlation was found between parameters, which describe albino and green plant regeneration/final production ability.

Discussion

Factors determining the efficiency of green and albino plant regeneration

The interest in DHs as the models for basic research and its practical exploitation in breeding programs is still growing. Due to that, a lot of effort has been put in an identification of the most important factors controlling androgenic responsiveness and effective production of DH lines. Although, numerous research papers, review articles, and books covering DH techniques have been published over the last decades, there are still some limitations in deployment of this technology for many important crop species. To overcome this problem, new molecular methods, which use marker genes associated with a specific trait, could be very helpful. One of the most important advantage of QTLs analysis is the possibility to identify genes of relatively small effects that do not produce individually recognizable phenotypes (Thomas et al. 2003). Another benefit comes from the ability to distinguish between stable QTLs detected in different genotypes/environments/seasons from those which reveals high level of variation resulting from QTL × environment × genotype interactions. This, seems to be very important as the quality of donor plant and conditions of its growth could significantly influence the final effectiveness of DH production (Datta 2005).

In triticale, low regeneration ability and high frequency of albino regenerants are the main limitations to incorporation of DHs into breeding programs (González et al. 1997; Mozgova et al. 2012; Pauk et al. 2000; Ponitka et al. 1999). Without chlorophyll, the primary pigment involved in light harvesting and its transformation into chemical energy, albinotic plants cannot survive in natural environment, and do not represent any agronomic value. Tuvesson et al. (2003) reported that albino plantlets have outnumbered green regenerants 2.6-fold, whilst in other studies, albinos have represented 42 % of all regenerated plantlets (Warzecha et al. 2005). In the main experiment presented, the mean number of regenerating AS gained 18.5 % with the frequency of albino plants at about 53 %. Even worse results were received in additional experiment performed on ten selected DH lines (6.3 % of regenerating AS among which 40 % regenerated albino plants).

In some cases, QTLs connected with albino plants were significantly conjugated with loci associated with green regenerants what explain strong correlations detected between studied variables by (Muñoz-Amartíain et al. 2008). In our study however, the correlation between green and albino plants formation was not found suggesting that the cellular mechanisms that control these processes are different. Similarly, González et al. (2005) and He et al. (1998) also revealed no correlation between green and albino plant regeneration in triticale and rice anther cultures, respectively.

Rather low variation between responsive DH lines in respect of regeneration parameters explain the fact that the majority of detected associations concerns recalcitrant genotypes. However, as the number of produced AS was in this group of genotypes usually very low, high variation in regeneration parameters does not mean the same in respect of absolute values. Another question is if found correlations signalize a real causal link between tested parameters. Despite all these concerns some interesting conclusions can be drawn from received data.

First, drastic change in detected associations observed as result of LT tillers treatment confirmed earlier hypothesis (Žur et al. 2014, 2015) that this stress factor stimulate androgenesis by affecting cellular redox status and hormonal balance (Baek and Skinner 2012; Bonnecarrere et al. 2011; Guo et al. 2006). Among ROS, H$_2$O$_2$ seems to be directly involved in androgenesis initiation (Žur et al. 2014), proper AS formation and plant regeneration. Without LT, osmotic stress induced by anther isolation acts as the signal stimulating microspore reprogramming. However, too high H$_2$O$_2$ generation diminish cell viability, so microspore survival and further proper development depends on the antioxidative system efficiency. Correlation analysis suggests also distinct difference in the role of two H$_2$O$_2$-decomposing enzymes: positive effect of CAT, stimulating GR frequency in anther cultures of responsive DH lines and negative effect of POX stimulating albino and detrimental for green plant regeneration efficiency in
It is well known that chloroplast biogenesis is controlled by cytokinins (CKs) and exogenously applied CKs can stimulate development of chloroplasts from proplastids, amyloplasts, and etioplasts (after Polanska et al. 2007). It has been revealed that this effect was mediated by up-regulation of the expression of some plastid-related genes, both of plastid and nuclear origin. Among them were the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) and chlorophyll a/b binding protein (Parthier 1989).

This group of hormones is involved also in almost all aspects of chloroplasts function: ultrastructure remodelling, enzyme activities, accumulation of photosynthetic pigments and photosynthetic activity (Zubo et al. 2008). In this experiment however, in anthers of recalcitrant DH lines isolated without LT treatment, GR regeneration and final production effectiveness were negatively correlated with prevailing form of cytokinins (cZ, cZR) detected in triticale anthers (Zur et al. 2015). On the contrary these plant hormones positively correlated with AR regeneration/final production effectiveness. Similarly, the presence of various anomalies in the ultrastructure of chloroplasts, from transgenic tobacco was the result of overproduction of endogenous CKs (Synkova et al. 2006). Surprisingly, after LT the same type of CKs positively correlated with GR regeneration/final production, whereas trans isomers of Z and ZR seems to have positive effect on AR regeneration/final production effectiveness. As LT increased endogenous content of these phytohormones in anthers of studied genotypes of triticale (Zur et al. 2015) some distinct changes in hormone reception/signaling pathways could be suspected.

In anthers of responsive DH lines isolated from freshly cut tillers (FC), no correlation was found between hormone concentration and the regeneration ability of produced AS. Disturbed hormone balanced induced by LT resulted in negative associations between ABA and GR regeneration and IAA and albino plant regeneration/final production effectiveness. It is well known that in plants, ABA plays a key role in stress response and also together with cytokinins take part in the development of the photosynthetic apparatus (Kraftsov et al. 2011). Just recently, trying to overcome the problem of albinism in microspores and anther culture of horse chestnut Calic et al. (2013) found that addition of low...
concentration (0.01 mg l\(^{-1}\)) of ABA to media reduced this obstacle. On the other hand, high concentration of ABA (8 mg l\(^{-1}\)) in media decreased the induction and regeneration ability of calluses obtained from wheat mature embryos (Fazeli-nasab et al. 2012). Previous results, where weak/moderate but significant negative correlation between the concentration of ABA in triticale anthers and parameters of regeneration efficiency was found (Żur et al. 2012) were confirmed only fragmentary in presented study as ABA seems to diminished albino plant regeneration. As in response to exogenous ABA application increased level of hydrogen peroxide (H\(_2\)O\(_2\)) and increased antioxidative system activity were observed (Agarwal et al. 2005; Jiang and Zhang 2001) it could be the mechanism of indirect ABA involvement in triticale microspore embryogenesis.

Localization of genomic regions controlling albino plant regeneration

One way to overcome the problem of albinism is the localization of the regions and identification of genes which control the formation of chlorophyll-deficient plants both in plastid and nuclear genome. Although, large defects in the pDNA structure are often detected among albino plants regenerated, it cannot be treated as the primary cause of albinism. Changes were also identified in the transcription levels of the nucleus-coded chloroplast-localized proteins (Dunford and Walden 1991) what indicates the important role of the nuclear genome in proper plastid function. Based on crosses between albinism-susceptible and albinism-resistant cultivars, it has been concluded that the genes responsible for this character are inherited in a Mendelian fashion and hence, they must be nuclear encoded (Larsen et al. 1991). Moreover, recent studies confirmed that the phenomenon of albinism is genetically determined (Clément et al. 2005; Kravtsov et al. 2011; Muñoz-Amatriain et al. 2008) and that the chloroplast development in hybrid genotypes was mostly influenced by nuclear factors (Kumari et al. 2011). Therefore, studies focused on nuclear genome, for example on the identification of genetic loci associated with the ratio of green to albino regenerants obtained via androgenesis seems to be very reasonable.

Majority of studies aimed at the identification of QTLs controlling the regeneration phase of androgenesis have been focused on the ability to form green plants. The works on wheat, barley and rice have identified many different genome regions affecting this trait (Chen et al. 2007; Grosse et al. 1996; Manninen 2000; Torp et al. 2001). Localization of genomic regions associated with green plant regeneration ability in triticale has been reported by González et al. (2005) and Krzewzska et al. (2012). Only limited number of papers focused on QTLs associate with albino plant regeneration and in all of them barley have been used as the plant model (Bregitzer and Campbell 2001; Muñoz-Amatriain et al. 2008).

In presented study, QTL analysis by using both methods—SMA and CIM identified 14 chromosomal regions among subgenome B (4 QTLS) and R (10 QTLS) involved in albino plants formation during anther culture in winter triticale (Fig. 2). The biggest number of QTLS connected with AR/100A were localized in subgenome R and the positive effect originated from ‘Modus’ allele. It could be connected with general recalcitrance in regard to the in vitro culture response in rye (Ma et al. 2003; Ma and Pulli 2004; Targańska et al. 2013). Generally problems associated with rye anther and microspore culture are poor embryogenic callus induction, low green plant regeneration and high proportion of albinos (Ma and Pulli 2004). The lack of response was found to be controlled by at least two interacting genes (Rakoczy-Trojanowska and Malepszy 1995). Bolibok et al. (2007) reported that nine putative QTLS for rye tissue culture response have been mapped on chromosomes 1R, 4R, 5R, 6R and 7R. In our study four of QTLS connected with albino plants formation were found on chromosome 4R. Moreover, one QTL QARS\(_{sm4R}\)-4 was located in the same region as QTL associated with final green plant regeneration ability—QGR\(_{sm4R}\)-2 (Krzewska et al. 2012). Three of them: QARS\(_{sm4R}\)-1, QARS\(_{sm4R}\)-2, QARS\(_{sm4R}\)-3 were conjugated with QTLS controlling final regeneration ability (Krzewska et al. 2012). Similar results were received by González et al. (2005), who also detected QTLS for final efficiency of green plants regeneration on chromosome 4R on triticale genome. Moreover, callus induction and somatic embryogenesis ability in rye tissue cultures are also determined by loci located on this chromosome (Bolibok et al. 2007).

Three regions on chromosome 5R had a major effect on albino plant regeneration ability. One of them QARS\(_{sm5R}\)-1 with high LOD value (5.6) explains almost 17 % of phenotypic variation. Earlier
reports revealed that QTLs controlling green plant formation are located on chromosome 5R in rye (Grosse BA, Deimling S, Geiger HH Mapping of genes for anther culture ability in rye by molecular markers. In: Vortr P?anzenzuechtg1996), 5B in wheat (Torp et al. 2001) or 5H in barley (Bregitzer and Campbell 2001; Muñoz-Amatriaín et al. 2008). This could indicate a possible role of cereal group 5 chromosomes in the control of green and albino plant regeneration in tissue culture.

According to our research, the triticale chromosome 7R seems to be connected with albino plant regeneration as well as with final efficiency of androgenesis (González et al. 2005; Krzewska et al. 2012). QTL $QAR_{sm}$-7R was mapped in the same chromosome region as $QR_{sm}$-7R-1 controlling the total number of regenerants per 100 anthers (Krzewska et al. 2012). Bolibok et al. (2007) found on this chromosome locus influencing callus induction in the culture of rye immature inflorescences. What is more, chromosomes: 3RS, 4RL and 5RL in rye are identified as carrying the QTLs affecting chlorophyll content (Milczarski and Masojc 2002). The locus $QChc_{-}3R.1$ had approximate position as QTL $QAR_{sm}$-2RS.3R connected with albino plant regeneration in our study.

SMA revealed that one of DArT markers (rPt-399503) on chromosome 2RS.3R was significantly associated with QTL for AR/100A. Other authors have reported the involvement of loci on this chromosome in the green plant regeneration capacity from microspores in triticale (González et al. 2005), the photosynthetic viability and general androgenesis process in rye (Grosse et al. 1996).

A few regions in subgenome B in triticale seem to control the regeneration yield during androgenesis. However, QTLs located in wheat subgenome had minor effects on studied traits and all positive alleles came from maternal genotype ‘Saka 3006’. Torp et al. (2001) identified three QTLs for green plant percentage on chromosomes: 2AL, 2BL and 5BL in DH mapping population of wheat. Other studies on wheat also revealed QTLs associated with green plant regeneration on chromosome 5BL (Zhang et al. 2003) and on chromosomes 2A and 2B (Anca et al. 2007).

Our result did not confirm the existence of QTL on chromosome 5B responsible for green or albino plant regeneration, but we found one QTL on chromosome: 2AL, 2BL and two QTLs on chromosome 3B associated with albino plant formation ability (AR/100AS). Moreover, QTL analysis identified one chromosomal region for AR/100A with high LOD score (4.8), explaining up to 17.6 % of the phenotypic variance on chromosome 4B.

QTL analysis seems to prove ROS and ABA involvement in androgenesis regulation (Fig. 2). It occurred that one chromosomal region controlling AntiOx activity localized on chromosome 4A (data not published) was conjugated with locus $QAR_{sm}$-4A-1 associated with total regeneration ability in triticale (Krzewska et al. 2012) and another QTL on chromosome 5R connected with antioxidative activity (data not published) was mapped close to $QAR_{sm}$-5R-1 responsible for albino plants regeneration.

Additionally, some QTLs associated with ABA accumulation in triticale anthers (Zur et al. 2012) were localized on the same chromosomes as QTLs connected with albino plant formation (Fig. 2). The example could be QTL $QAR_{sm}$-2RS.3R was conjugated with marker rPt-509415 significantly associated with QTL for endogenous level of ABA whereas $QAr_{ch}sm$-5R was mapped on chromosome 5R the same as loci $QAR_{sm}$-5R-3. It could be supposed that close localization of QTLs associated with in vitro embryogenesis, ABA and antioxidative system activity is not meaningless and suggests a complex network of interactions.

In conclusion, the major QTLs controlling albino plant creation are located on rye chromosomes, where the positive effect was originated from ‘Modus’ allele. QTLs mapped on chromosomes B had minor effect on studied trait and show a positive effect when the allele comes from maternal genotype.

The analysis of correlations found between albino/green plant regeneration ability, hormonal balance and antioxidative system activity confirmed earlier hypothesis (Zur et al. 2014, 2015) that LT stimulate androgenesis by affecting cellular redox status and hormonal balance. It seems that in anthers isolated without LT treatment, some critical level of H$_2$O$_2$ generation was a prerequisite for successful transition from a non-photosynthetic proplastid to a functional chloroplast. LT treatment seems to change the main importance for effective androgenesis induction from redox status to endogenous hormonal composition of the anthers.
The presented results made the next step in widening the knowledge of molecular background of albino plant formation during androgenesis in winter triticale. Markers identified as linked with QTLs controlling studied traits could help breeders in the selection of best genotypes and optimizing the inputs needed for DH production. Nonetheless, obtained results should be confirmed and validated in different genetic backgrounds, by testing the reliability of markers associated with QTLs to predict phenotype.

Acknowledgments The research was conducted in the frame of the COST Action FA0604 ‘Triticaceae genomics for the advancement of essential European crops (TritiGen)’, supported by the polish Ministry of Science and Higher Education (research project 548/N-COST/2009/0) and by National Science Centre (NCN); Project No. 2011/01/N/NZ9/02541.

Author contribution MK co-performed the experiments, co-analyzed the data and co-wrote the manuscript. ICz-M, ED, GG-P performed the experiments and collected the raw data. IŻ designed the experiments, co-analyzed the data and co-wrote the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

Agarwal S, Sairam RK, Srivastava GC, Tyagi A, Meena RC (2005) Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings. Plant Sci 169:559–570. doi:10.1016/j.plantsci.2005.05.004

Anca M, Andersen SB, Banga D, Ardelean M, A-m Torp (2007) Genetic mapping of wheat 2BL chromosome using SSR markers. Bull Univ Agric Sci Veterinary Med Cluj-Napoca 64:48–53

Andersen SB, Due IK, Olesen A (1987) The response anther culture in a genetically wide material of winter-wheat (Triticum aestivum L.). Plant Breed 99:181–186. doi:10.1111/j.1439-0523.1987.tb01170.x

Ankele E, Heberle-Bors E, Pfosser MF, Hofinger BJ (2005) Searching for mechanisms leading to albino plant formation in cereals. Acta Physiol Plant 27:651–664

Asif M, Eudes F, Goyal A, Amundsen E, Randhawa H, Spaner D (2013) Organelle antioxidants improve microspore embryogenesis in wheat and triticale. In Vitro Cell Dev Biol 49:489–497. doi:10.1007/s11627-013-9514-z

Baek K-H, Skinner DZ (2012) Production of reactive oxygen species by freezing stress and the protective roles of antioxidant enzymes in plants. J Agric Chem Environ 01:34–40. doi:10.4235/jacen.2012.1.11006

Bolíbok H, Gruszczynska A, Hromadja-Judycya A, Rakoczyc-Trojanowska M (2007) The identification of QTLs associated with the in vitro response of rye (Secale cereale L.). Cell Mol Biol Lett 12:523–535. doi:10.2478/s11658-007-0023-0

Bonet FJ, Azbaid L, Olmedilla A (1998) Pollen embryogenesis: atavism or totipotency? Protoplasma 202:115–121. doi:10.1007/bf01282539

Bonncarevra V, Borsani O, Díaz P, Capdevielle F, Blanco P, Monza J (2011) Response to phototoxic stress induced by cold in japonica rice is genotype dependent. Plant Sci 180:726–732. doi:10.1016/j.plantsci.2011.01.023

Bregitzer P, Campbell RD (2001) Genetic markers associated with green and albino plant regeneration from embryogenic barley callus. Crop Sci 41:173–179

Čalić D, Bohanec B, Devrnja N, Miolojević J, Tukić L, Kostić I, Zdravković-Korač S (2013) Impact of abscisic acid in overcoming the problem of albinism in horse chestnut androgenic embryos. Trees 27:755–762. doi:10.1007/s00468-012-0830-4

Caredda S, Doncoeur C, Devaux P, Sangwan RS, Clement C (2000) Plastid differentiation during androgenesis in albino and non-albino producing cultivars of barley (Hordeum vulgare L.). Sex Plant Reprod 13:95–104. doi:10.1007/s00470000043

Caredda S, Devaux P, Sangwan RS, Proult I, Clément C (2004) Plastid ultrastructure and DNA related to albinism in androgenetic embryos of various barley (Hordeum vulgare) cultivars. Plant Cell Tissue Organ Cult 76:35–43

Chatterjee M, Sparvoli S, Edmunds C, Garosi P, Findlay K, Martin C (1996) DAG, a gene required for chloroplast differentiation and palisade development in Antirrhinum majus. EMBO J 15:4194–4207

Chen X-W, Cistué L, Muñoz-Amatriain M, Sanz M, Romagosoa I, Castillo A-M, Vallés M-P (2007) Genetic markers for doubled haploid response in barley. Euphytica 158:287–294. doi:10.1007/s10681-006-9310-5

Clément C, Sangwan RS, Sangwan-Norreel B (2005) Microspore embryo induction and development in higher plants: cytological and ultrastructural aspects. In: Palmer CE, Keller WA, Kashia KJ (eds) Haploids in crop improvement II. Springer, Berlin, pp 53–72

Dat J, Vandenabeele S, Vranova E, Van Montagu M, Inze D, Van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci 57:779–795

Datta SK (2005) Androgenic haploids: factors controlling development and its application in crop improvement. Curr Sci 89:1870–1878

Dunford R, Walden RM (1991) Plastid genome structure and plastid-related transcript levels in albino barley plants derived from anther culture. Curr Genet 20:339–347. doi:10.1007/bf00318524
Kravtsov AK, Zubo YO, Yamburenko MV, Kulaeva ON, Knudsen S, Due IK, Andersen SB (1989) Components of androgenesis of triticale in isolated microspore culture. Plant Cell Rep 31:2099–2108. doi:10.1007/s00299-012-1320-2

Kumari M, Clarke HJ, des Francs-Small CC, Small I, Khan TN, Siddique KH (2011) Albinism does not correlate with biparental inheritance of plastid DNA in interspecific hybrids in Cicer species. Plant Sci 180:628–633. doi:10.1016/j.plantsci.2011.01.003

Lantos C, Böna L, Boda K, Pauk J (2013) Comparative analysis of in vitro anther- and isolated microspore culture in hexaploid Triticale (× Triticosecale Wittmack) for androgenic parameters. Euphytica 197:27–37. doi:10.1007/s10681-013-1031-y

Larsen ET, Tuveson IKD, Andersen SB (1991) Nuclear genes affecting percentage of green plants in barley (Hordeum vulgare L.) anther culture. Theor Appl Genet 82:417–420

Ma R, Pulli S (2004) Factors influencing somatic embryogenesis and regeneration ability in somatic tissue culture of spring and winter rye. Agric Food Sci 2008(13):363–377

Ma R, Guo YD, Pulli S (2003) Somatic embryogenesis and fertile green plant regeneration from suspension cell-derived protoplasts of rye (Secale cereale L.). Plant Cell Rep 22:320–327. doi:10.1007/s00299-003-0694-6

Makowska K, Oleszczuk S (2014) Albinism in barley androgenesis. Plant cell reports 33:385–392. doi:10.1007/s00299-013-1543-x

Manninen OM (2000) Associations between anther-culture response and molecular markers on chromosomes 2H, 3H and 4H of barley (Hordeum vulgare L.). Theor Appl Genet 100:57–62. doi:10.1007/s001220050008

Milczarski P, Masojc P (2002) The mapping of QTLS for chlorophyll content and responsiveness to gibberellic (GA3) and abscisic (ABA) acids in rye. Cell Mol Biol Lett 7:449–455

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Mozgovaya G, Zaitseva O, Lemes V (2012) Structural changes in chloroplast genome accompanying albinism in another culture of wheat and triticale. Cereal Res Commun 40:467–475. doi:10.1556/CRC.2012.0007

Muñoz-Amatriain M, Castillo AM, Chen XW, Cistué L, Vallés MP (2008) Identification and validation of QTLS for green plant percentage in barley (Hordeum vulgare L.) anther culture. Mol Breed 22:119–129. doi:10.1007/s11032-008-9161-y

Muñoz-Amatriain M, Svensson JT, Castillo AM, Close TJ, Valles MP (2009) Microspore embryogenesis: assignment of genes to embryo formation and green vs. albino plant production. Funct Integr Genomics 9:311–323. doi:10.1007/s10142-009-0071-3

Parthier B (1989) Hormone-induced Alterations in Plant Gene Expression. Biochem Physiol Pflanzen 185:289–314. doi:10.1016/S0015-3796(89)80051-4

Pauk J, Puolimatka M, Toth KL, Monostori T (2000) In vitro androgenesis of triticale in isolated microspore culture. Plant Cell Tissue Organ Cult 61:221–229. doi:10.1023/a:1006416116366
Polanska L et al (2007) Altered cytokinin metabolism affects cytokinin, auxin, and abscisic acid contents in leaves and chloroplasts, and chloroplast ultrastructure in transgenic tobacco. J Exp Bot 58:637–649. doi:10.1093/jxb/erl235

Ponitka A, Slusarkiewicz-Jarzina A, Wozdny M, Marcinska I, Wozny J, Marcinska I, Wozna J (1999) The influence of various in vitro culture conditions on androgenetic embryo induction and plant regeneration from hexaploid triticale (*Triticosecale Wittm.*). J Appl Genet 40:165–174

Rakoczy-Trojanowska M, Malepszy S (1995) Genetic factors influencing the regeneration ability of rye (*Secale cereale* L.) II. Immature embryos. Euphytica 83:233–239. doi: 10.1007/BF01678135

Rohde A, De Rycke R, Engler G, Van Montagu M, Rakoczy-Trojanowska M, Malepszy S (1995) Genetic factors associated with abscisic acid accumulation in triticale (*Triticosecale Wittm.*) anther cultures. Plant Cell Rep 13:35–52

Schumann G (1990) In vitro production of haploids in triticale. In: Bajaj YPS (ed) Wheat. Biotechnology in agriculture and forestry, vol 13. Springer, Berlin, pp 382–402. doi: 10.1007/978-3-662-10933-5_19

Segui-Simarro JM (2010) Androgenesis revisited. Bot Rev 76:377–404. doi:10.1007/s12229-010-9056-6

Ślusarkiewicz-Jarzina A, Ponitka A (1997) Effect of genotype and media composition on embryo induction and plant regeneration from anther culture in triticale. J Appl Genet 38:253–258

Synkova H et al (2006) Three-dimensional reconstruction of anomalous chloroplasts in transgenic ipt tobacco. Planta 223:659–671. doi:10.1007/s00425-005-0119-6

Szechynska-Hebda M et al (2011) Identifying QTLs for cold-stress–induced malformation of microspore calli. Plant Physiol Biochem 117:851–858

Targonska M, Hromada-Judycka A, Bolibok-Bragoszewski H, Rakoczy-Trojanowska M (2013) The specificity and genetic background of the rye (*Secale cereale* L.) tissue culture response. Plant Cell Rep 32:1–9. doi:10.1007/s00299-012-1342-9

Tewari AK, Tripathy BC (1998) Temperature–stress–induced impairement of chlorophyll biosynthetic reactions in cucumber and wheat. Plant Physiol Biochem 117:851–858

Thomas WTB, Forster BP, Gertsson B (2003) Doubled haploids in breeding. In: Maluszynski M, Kasha KJ, Forster BP, Szarejko I (eds) Doubled haploid production in crop plants. Springer, Dordrecht, pp 135–140. doi:10.1007/978-1-4020-2841-1_21

Tyrka M, Bednarek PT, Kilian A, Wdzydzy M, Hura T, Bauer E (2011) Genetic map of triticale compiling DArT, SSR, and AFLP markers. Genome 54:391–401. doi:10.1139/g11-009

Wang P, Chen Y (1983) Preliminary study on prediction of height of pollen H2 generation in winter wheat grown in the field. Acta Agron Sin 9:283–284

Wang S, Basten CJ, Zeng Z-B (2012) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh

Wojnarowicz G, Jacquard C, Devaux P, Sangwan RS, Clement C (2002) Influence of copper sulfate on anther culture in barley (*Hordeum vulgare* L.). Plant Sci 162:843–847. doi:10.1016/s0168-9452(02)00036-5

Zhang LY, Xu J (1983) Increasing differentiation frequencies in wheat pollen callus. In: Hu H, Vega MR (eds) Cell and tissue culture techniques for cereal crop improvement. Science Press, Beijing, pp 431–432

Zhang LY, Sourdille P, Bernard M, Madeore A, Bernard S (2003) QTL mapping for anther culturability in wheat using a doubled-haploid population. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proceedings of the 10th international wheat genetic symposium, Paestum, 1–6 September 2003. pp 1078–1080

Zhou H, Konzak CF (1992) Genetic control of green plant regeneration from anther culture of wheat. Genome 35:957–961. doi:10.1139/g92-146

Zubo YO et al (2008) Cytokinin stimulates chloroplast transcription in detached barley leaves. Plant Physiol 148:1082–1093. doi:10.1104/pp.108.122275

Żur I, Krzewska M, Dubas E, Golebiowska-Pikania G, Janowiak F, Stojalowski S (2012) Molecular mapping of loci associated with abscisic acid accumulation in triticale (*Triticosecale Wittm.*) anthers in response to low temperature stress inducing androgenic development. Plant Growth Regul 68:483–492. doi:10.1007/s10725-012-9738-7

Żur I et al (2014) Antioxidant activity and ROS tolerance in triticale (*Triticosecale Wittm.*) anthers affect the efficiency of microspore embryogenesis. Plant Cell Tissue Organ. doi:10.1007/s11240-014-0515-3

Żur I, Dubas E, Krzewska M, Waligórska P, Dziurka M, Janowia F (2015) Hormonal requirements for effective induction of microspore embryogenesis in triticale (*Triticosecale Wittm.*) anther cultures. Plant Cell Rep 34:47–62. doi:10.1007/s00299-014-1686-4