Induction of in vitro androgenesis in anther culture of recalcitrant einkorn (*Triticum monococcum* L.)

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Abstract

Einkorn (*Triticum monococcum* L.) can be applied as a model species for cereal genomic studies due to its small genome size and high level of polymorphism. The in vitro somatic tissue culture protocol in einkorn was significantly improved recently, however the in vitro androgenesis remained an unresolved research topic. Five different pre-treatments were compared to study the effects of stress pre-treatments on the efficiency of androgenesis in two einkorn genotypes. The long cold pre-treatment (2 weeks, 4 °C) of donor tillers increased significantly the number of microspore derived embryo-like structures (ELS). Green and albino plantlets were regenerated from these structures. The ploidy level of microspore-derived green plantlet was determined as haploid by flow cytometric analyses. This is the first report published on the successful androgenesis induction (ELS production) and green- and albino plantlet regeneration in in vitro anther culture of the recalcitrant einkorn wheat (*Triticum monococcum* L.).

Key message

This publication reported at first the induction of androgenesis (ELS, green and albino plantlets production, flow cytometry) in in vitro anther culture of einkorn (*Triticum monococcum* L.).

Keywords

Einkorn · *Triticum monococcum* L. · Androgenesis · Anther culture · Flow cytometry · Haploid

Abbreviations

AC  Anther culture
DH  Doubled haploid,
ELS  Embryo-like structures

Introduction

Einkorn (*Triticum monococcum* L.) is an ancient diploid wheat which is frequently used in different researches and crop breeding programmes. This plant species is dominantly grown in marginal regions of agricultural lands, in high mountains as a component of pasture (in Transylvania) and its popularity is growing in organic farming. Einkorn is cultivated recently in some poor soils of Southern Europe, Minor Asia, Caucasus, North Africa (Miroshnichenko et al. 2017). As a functional food, this diploid cereal has many benefits over the cultivated modern tetraploid and hexaploid wheat varieties, such as higher level of macro- and micronutrients (phosphorus, sulfur, magnesium, zinc, manganese etc.) and various antioxidant (conjugated polyphenols, carotenoids, tocots, alkylresorcinols, and phytosterols) compounds (Suchowilska et al. 2012; Zaharieva and Monneveux 2014; Arzany and Ashraf 2017). Furthermore, einkorn can be used in modern wheat breeding programmes as a source of new traits for pest and disease resistance and abiotic stress tolerance (Zaharieva and Monneveux 2014; Longin et al. 2016; Miroshnichenko et al. 2017). Hence einkorn is in the focus of research, breeding and crop production of *Triticum spp*.

Einkorn has been applied as a model species for wheat genomic studies due to its small genome size, high level of polymorphism and easy cultivation procedure (Jing et al. 2007; Miroshnichenko et al. 2017) and agronomically important genes (*Lr10, VRN1, VRN2, NAC* gene family etc.)
were identified and mapped (Stein et al. 2000; Feuillet et al. 2003; Yan et al. 2003, 2004; Uauy et al. 2006). The application of modern biotechnological methods (RNAi based gene silencing, chloroplast transformation, genome editing, in vitro selection) require efficient in vitro plant regeneration protocols.

The in vitro somatic and haploid plant production protocols are widely utilised methods in hexaploid Triticum spp. such as bread wheat, spelt wheat or triticale (Purnhauser and Gyalai 1993; Kümlehn and Hensel 2009; Wuereschum et al. 2012; Castillo et al. 2015; Jiang et al. 2017; Lantos et al. 2019; Testillano 2019; Lantos and Pauk 2020; Niazian and Shariatpanahi 2020). Notwithstanding that most of the publications described the einkorn as a recalcitrant species in regards to the aspect of in vitro cell and tissue culture, some of the latest well-established somatic tissue culture protocols shows success (Alkina et al. 2016; Miroshnichenko et al. 2017, 2018; Agil et al. 2021; Orgec et al. 2021). An efficient method was established by the accurate improvements of critical factors (explant type, developmental stage of explant and combination of plant growth regulators) in somatic tissue culture of einkorn (Miroshnichenko et al. 2017). Furthermore, this protocol was applied for genetic transformation (Miroshnichenko et al. 2018) which results have opened a new scientific topic in research of diploid Triticum monococcum L. However, the in vitro androgenesis induction remained an unresolved research topic in einkorn tissue culture till now.

The increasing importance of doubled haploid (DH) plant production methods are incontrovertible in modern plant breeding and research programmes of crop plants. These methods such as chromosome elimination, anther culture (AC) and isolated microspore culture serve the quickest way for production of homozygous lines to accelerate the plant breeding and applied research. Furthermore, these methods were combined with other biotechnological approaches such as marker assisted selection (MAS), QTL analyses, genetic transformation or induced mutation to get the desired goals of crop breeding and research (Chauhan and Khurana 2011; Wessels and Botes 2014; Barakat et al. 2017; Ren et al. 2017; Song et al. 2017; Shchukina et al. 2018; Tyrka et al., 2018; Shi et al., 2019; Testillano, 2019; Wajdzik et al., 2019; Bilichak et al., 2020).

Due to its many advantages, DH plant production methods are widely applied in cereal species, for example: barley, wheat, triticale, maize and rice (Dunwell 2010; Germana 2011; Hensel et al. 2012; Niu et al. 2014). In contrast improvement of haploid induction methods has not been previously reported in connection with einkorn. Plamenov et al. (2009) attempted to induce androgenesis in in vitro AC of einkorn, although callus or ELS production was not observed in their experiments. Thus, in vivo and in vitro haploid induction remained a challenge in einkorn.

The in vitro androgenesis (anther- and microspore culture) is influenced by many factors, such as genotype, growing conditions, collection time, pre-treatments, induction and regeneration media and culture conditions. The combinations of these factors determine the efficiency of in vitro AC (Datta 2005; Testillano 2019; Lantos and Pauk 2020; Niazian and Shariatpanahi 2020). The objective of this research was to study the efficiency of stress pre-treatments on the in vitro androgenesis in AC of einkorn genotypes. The effectiveness of five different pre-treatments were compared on the androgenic parameters in einkorn. The number of produced embryo-like structures (ELS), green and albino plantlets were statistically compared. The ploidy level of microspore-derived green plantlet was determined by flow cytometric analyses. This is the first report which has focused on the androgenesis induction (ELS production and plant regeneration) in AC of recalcitrant einkorn (Triticum monococcum L.) and identified a microspore-derived haploid green plant by flow cytometric analyses.

Materials and methods

Plant materials and growing conditions

Two winter growing type einkorn genotypes (‘G7026’ and ‘G7176’) were used as donor materials, which derived from our gene bank. The donor genotypes were grown following a normal European wheat nursery protocol in the nursery of Cereal Research Non-Profit Ltd., Szeged. The donor plants were fertilized (1 nitrogen: 1 phosphorus: 1 potassium) in autumn (12 g/m²), and 18 g/m² ammonium nitrate was added in mid-April. The donor plants were protected against insects by two insecticidal treatment. Weeds were controlled by mechanical treatment before sowing; and by chemical treatment and manual weeding during the vegetation period.

Collection of donor materials, pre-treatments

The microspore developmental stages in spikes were checked by Olympus CK-2 inverted microscope (Olympus Ltd., Southend-on-Sea, UK) and the donor tillers were collected when the microspores were in early- and mid-uninucleate stages in anthers for in vitro induction of androgenesis. Five different treatments were applied to compare the effectiveness of pre-treatments on androgenesis induction in einkorn genotypes (Table 1).

In treatment 1, the anthers of collected materials were isolated directly to the pre-treatment medium (Cistué et al. 2003), and the isolated anthers were pre-treated at 24 °C for 4 days in dark thermostat. In treatment 2, the dissected spikes with two drops tap water were placed into Petri dishes and stored at 4 °C for 5 days. In the following
treatments (treatment 3, 4, 5), the donor tillers were collected into Erlenmeyer flasks containing tap water and covered by a PVC bag to keep high humidity and stored at 2–4 ºC (Pauk et al. 2003; Lantos et al. 2013; Lantos and Pauk 2016; Coelho et al. 2018; Wang et al. 2019). In treatment 3, the donor tillers were cold pre-treated for 9 days, following by 5 days 4 ºC treatment of the dissected spikes. The donor tillers were only cold pre-treated for 14 days in treatment 4 and 3 days heat shock were applied after the 14-days cold period in treatment 5. (Ouyang et al. 1983; Pauk et al. 2003; Shariatpanahi et al. 2006; Lantos et al. 2013; Lantos and Pauk 2016).

### Sterilization of donor materials and preparation of AC

The awns of the selected spikes were cut by scissors to facilitate the isolation of anthers. Before each isolation, the donor spikes were placed in 150 ml 2% NaO’Cl solution with one drop Tween-80. The solution with spikes were agitated for 20 min on a shaker. After the sterilization, the donor spikes were rinsed three times with distilled water (Millipore Elix 5) in clean bench.

Anthers of donor genotypes were isolated in 60 mm diameter glass Petri dishes (100 anthers/Petri dish) containing 5 ml W14mf induction medium (Ouyang et al. 1989; Lantos and Pauk 2016). In W14mf, W14 basic medium was supplemented with 90,000 mg/l maltose, 2 mg/l 2,4-D, 0.5 mg/l kinetin, 100,000 mg/l Ficoll and pH was adjusted at 5.8 (Lantos and Pauk 2016). Following the different pre-treatments, each Petri dish was incubated at 28 ºC for 8 weeks in darkness.

### Plant regeneration and acclimatization

The AC-derived ELS with a size of 1–2 mm were transferred into 90 mm diameter plastic Petri dishes (Sarstedt, Newton, MA, USA), which contained 190-2Cu regeneration medium (Pauk et al. 2003). The number of albino and green plantlets were registered. The structures were placed week by week on the regeneration medium (approximately 30–40/Petri dish). The regenerated albino plantlets were discarded, while the in vitro green plantlet was transferred into a glass tube, which contained the 190-2Cu regeneration medium for rooting.

The well-rooted green plantlet was transferred to the greenhouse, where it was transplanted into a plastic pot containing a 1:1 peat and sandy soil mixture and covered by a PVC bag for a one week acclimatization period. Following the acclimatization, the transplanted plantlet was grown in the greenhouse under a standard cereal growing condition.

### Flow cytometric analyses

The ploidy level of the acclimatized green plantlet and donor plants was determined by flow cytometric analyses using the CytoFLEX Flow Cytometer (Beckman Coulter International S.A., Nyon, Switzerland). The leaf samples (100 mg/plant) were collected from young leaves of plantlets grown in the greenhouse.

The leaf samples were homogenized in Eppendorf tubes containing 1 ml Galbraith puffer and two stainless steel beads using TissueLyser II (Qiagen GmbH., Hilden, Germany) at 20 Hz for 1 min (Galbraith et al. 1983). After the suspensions were purified using 20 μm sieves, 10 μl RNase solution was added to each sample for 60 min to eliminate
the RNA content. Before flow cytometry, DNA content was stained with 40 µl PI solution (1 mg/ml) for 30 min. The ploidy levels of leaf samples were determined based on the histograms of flow cytometric analysis.

**Statistical analyses**

Our experiments were carried out at least with four replications. The most important parameters of in vitro androgenesis (number of ELS, regenerated green and albino plantlets) were collected and analysed by two-way ANOVA. The statistical analyses were carried out using Microsoft Excel 2013 statistical software developed by Microsoft Ltd. (Redmond, WA, USA).

**Results**

**Induction of in vitro androgenesis in AC of einkorn genotypes**

Prior to induction of androgenesis, the developmental stages of microspores from the donor spikelets were checked by Olympus CK-2 inverted microscope. The yellowish green spikes (Fig. 1a), containing dominantly uninucleate microspores (Fig. 1b), were selected from the donor spikes and used for the experiments. The in vitro androgenesis was induced in AC of both tested genotypes after the application of appropriate stress pre-treatments. The first AC-derived ELS were visible to the naked eye four-weeks after culture. The ELS with 1–2 mm size (Fig. 1c) were transferred to the regeneration medium, where the ELS regenerated plantlets (albino and green) within two weeks (Fig. 1d–e). Albinos were regenerated from ELS of both genotypes, while a single green plantlet was regenerated from a microspore-derived ELS of ‘G7176’ genotype, which was acclimatised to the greenhouse conditions (Fig. 1f).

**Study the effect of genotype, pre-treatments and their interaction in AC of einkorn genotypes**

Five different pre-treatments were compared using two einkorn genotypes in in vitro AC to analyse the effect of stress pre-treatments on androgenic parameters (number of ELS, albino and green plantlets). Based on statistical analyses (Table 2), the pre-treatment and genotype influenced significantly the number of ELS and albino plantlets. There was significant genotype × pre-treatment interaction on the number of albino plantlets. Significant differences were not found between the numbers of regenerated green plantlets by two-way ANOVA.

Androgenesis was induced in in vitro AC of both genotypes following all the applied pre-treatments except in treatment 1. ELS production was not observed after the application of 4-day starvation (treatment 1), while ELS were detected in the other treatments. The number of produced ELS were significantly different between the pre-treatments (Table 3). Significantly more ELS production was observed following treatment 3 and 4 in comparison with the treatment 2 and 5. The mean of ELS production was 77.25 ELS/100 anther in AC of ‘G7026’ following treatment 3, while the ‘G7176’ genotype produced 28.25 ELS/100 anthers after treatment 4.

The plant regeneration efficiency was mitigated from the microspore-derived ELS. The ELS of einkorn genotypes produced some albino plantlets following treatment 3 and 4 (Table 3). Altogether five albinos were regenerated from AC-derived ELS of ‘G7026’ genotype following treatment 3 where the donor tillers were cold pre-treated for 9 days with a subsequent 4 ºC treatment of the dissected spikes for 5 days. The ‘G7176’ genotype produced one albino plantlet after the donor tillers were cold pre-treated for 14 days (treatment 4). Furthermore a single green plantlet was regenerated from the microspore-derived structures of ‘G7176’ genotype after application of 14 days cold pre-treatment (treatment 4). The green plantlet was transplanted to the greenhouse where it developed as an einkorn plant. It was flowering similarly as plants of donor genotype, although it produced sterile spikes without any seed.

**Analyses of ploidy level by flow cytometry in einkorn**

The ploidy level of the regenerated green plantlet and a seed-grown diploid einkorn plant were compared by flow cytometric analyses to determine ploidy level of the AC-derived green plantlets. The measurement revealed the differences of DNA content among the tested samples (Fig. 2). The relative DNA content was two times higher in the leaf samples of control plant than in the sample of the regenerated green plantlet. Therefore the microspore origin (haploid) of the green plantlet was proved by flow cytometric analyses.

**Discussion**

Recently several research projects have been focused on the advancement of in vitro somatic tissue culture of einkorn wheat, consequently significant improvements were reported in this topic (Alikina et al. 2016; Miroshnichenko et al. 2017; Agil et al. 2021; Orgec et al. 2021). The published well-established protocols serve new biotechnological approaches, such as genetic transformation for applied research and breeding programmes (Miroshnichenko et al. 2018). However, the in vitro androgenesis induction remained a scientific challenge in einkorn tissue culture.
In vitro androgenesis is complex process in tissue culture of crop plants. For the successful AC induction the gametophyte pathway of microspore needs to be reprogrammed to sporophyte pathway by the application of different stress pre-treatments. Furthermore, the efficiency of in vitro androgenesis (AC and isolated microspore culture) is influenced by many factors, such as the genotype, growing conditions of donor plants, developmental stage of microspores, pre-treatments of the donor materials, compositions of induction and regeneration media and culture conditions. The combinations of these factors determine the efficiency of in vitro androgenesis (Datta 2005; Testillano 2019; Lantos and Pauk 2020; Niazian and Shariatpanahi 2020).

Stress pre-treatments play a key role in the induction of androgenesis. In *Triticum* spp., many stress factors were applied for the androgenesis induction, including cold,
heat, starvation, colchicine, osmotic shock, 2-HNA, DMSO etc. (Liu et al. 2001; Barnabás 2003; Shariatpanahi et al. 2006; Echávarri and Cistué 2016). Long cold pre-treatment, starvation and heat shock of donor materials are the most frequently applied stress pre-treatments in in vitro anther culture of *Triticum aestivum* L. (Ouyang et al. 1983; Cistué et al. 2003; Pauk et al. 2003; Shariatpanahi et al. 2006; Soriano et al. 2007, 2008; Sanchez-Diaz et al. 2013; Castillo et al. 2015; Echávarri and Cistué 2016). In in vitro AC of the tested einkorn genotypes, 4-day starvation of isolated anthers were applied in treatment 1 to induce androgenesis, but ELS formation was not observed in AC.

In re-programming of cereal microspores, one of the other most frequently applied stress pre-treatments is long cold pre-treatment (2–5 °C, 10 days–4 weeks) of donor tillers (Pauk et al. 2003; Lantos et al. 2013; Lantos and Pauk 2016; Coelho et al. 2018; Wang et al. 2019). In our experiment the highest values of ELS production was observed in AC of genotypes after long cold pre-treatment in both genotypes. Heat treatment (3 days, 32 °C) of isolated anthers in AC is a frequently applied stress factor (Ouyang et al. 1983; Pauk et al. 2003; Shariatpanahi et al. 2006; Lantos et al. 2013; Lantos and Pauk 2016), which enhances the efficiency of in vitro androgenesis responses. In this study 3 days 32 °C heat treatment did not increase the number of microspore-derived ELS in AC of einkorn after the same length of cold treatment (treatment 4 vs 5).

The genotype dependency of AC protocols is a widely published phenomenon in DH plant production of cereals (Lazar et al. 1984; Deaton et al. 1987; Agache et al. 1989; Tuvevsson et al. 2000; Lantos et al. 2019). In the present study, two einkorn genotypes were used to verify the effect of pre-treatments and study the genotype dependency and genotype × treatment interaction in einkorn AC. The different

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**Table 2** Study of effect of genotype, pre-treatments and their interactions on the efficiency of in vitro androgenesis (number of embryo-like structures (ELS), albino and green plantlets) in einkorn anther culture by two-way ANOVA

| Source          | df | MS of ELS     | MS of albinos | MS of green plantlets |
|-----------------|----|--------------|--------------|-----------------------|
| Pre-treatments  | 1  | 9517.225**   | 0.4**        | 0.025 ns              |
| Genotype        | 4  | 3443.838**   | 0.5875***    | 0.025 ns              |
| Pre-tr. × Gen   | 4  | 888.1625 ns  | 0.7125***    | 0.025 ns              |
| Error           | 30 | 725.825      | 0.05         | 0.025 ns              |

*ns* non-significant, *Pre-tr. × Gen.* Pre-treatment × Genotype interaction

***Significant at the 0.001 probability level

**Significant at the 0.01 probability level

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**Table 3** Effect of genotype and cold pre-treatments in anther culture of einkorn

| Genotype | Pre-treatment | ELS/100 anthers | Albinos/100 anthers | Green plantlets/100 anthers |
|----------|---------------|-----------------|--------------------|----------------------------|
| G7026    | 1             | 0.00 A c        | 0.00 A b           | 0.00 ns                    |
|          | 2             | 41.00 A b       | 0.00 A b           | 0.00 ns                    |
|          | 3             | 77.25 A a       | 1.25 A a           | 0.00 ns                    |
|          | 4             | 71.75 A a       | 0.00 A b           | 0.00 ns                    |
|          | 5             | 38.5 A b        | 0.00 A b           | 0.00 ns                    |
| G7176    | 1             | 0.00 A b        | 0.00 A a           | 0.00 ns                    |
|          | 2             | 8.25 B ab       | 0.00 A a           | 0.00 ns                    |
|          | 3             | 22.00 B ab      | 0.00 B a           | 0.00 ns                    |
|          | 4             | 28.25 B a       | 0.25 A a           | 0.25 ns                    |
|          | 5             | 15.75 B ab      | 0.00 A a           | 0.00 ns                    |

Values followed by the same capital letters (A, B, C) are not significantly (P = 0.05) different for the genotypes. Values followed by the same small letters (a, b) are not significantly (P = 0.05) different for different treatment within genotype

*ELS* embryo-like structures
statistical analyses revealed that the genotype influenced significantly the efficiency of AC (ELS and albinos) in einkorn and genotype × treatment interaction was significant on the number of albino plantlets. The green plantlet production was too low in this experiment to study the effect of genotype in this regard.

The conversion of microspore-derived ELS to plantlets is the next critical step in DH plant production methods, which is influenced by the above mentioned factors. The genotype dependency, albinism, low efficiency of green plant production are recognised as bottlenecks of successful use of AC in cereals (Li et al. 2013; Zhao et al. 2015, 2017; Wessels and Botes 2014; Weigt et al. 2020; Orlowska et al. 2020). The plant regeneration efficiency (green and albino) was low in AC of einkorn, a few albino and a single green plantlet were regenerated from the microspore-derived ELS. Even though this is the first publication which reported the plantlet (green and albino) production from AC-derived ELS of einkorn, further improvements are required to increase the efficiency of green plantlet production.

In cereals, flow cytometry is frequently applied method for characterization of ploidy level of the regenerated green plantlets. After some optimization (preparation of samples, parameters of measurement; data not shown), the flow cytometry was a useful tool to identify different ploidy levels in einkorn. Based on the histograms of flow cytometric analyses, the regenerated plantlet was proved as a haploid plant. After tillering, the regenerated haploid plantlet produced completely sterile spikes.

Genetic control of the tissue culture response is a well-known in in vitro androgenesis and somatic tissue culture of crop plants. In common wheat (Triticum aestivum L.), some experiments were implemented to identify the responsible chromosomes, QTLs which influence the ELS production and regeneration of green and albino plantlets in in vitro cell and tissue cultures. Several chromosomes (7B, 7D, 1D, 1B) were described which are responsible for plant regeneration efficiency in somatic tissue culture of wheat (Galiba et al. 1986; Henry and De Buyser 1985; Henry et al. 1994). AC of common wheat, more research groups reported that 1B, 1D, 2A, 2D, 4A, 4B, 5A, 5B and 7A chromosomes influenced the ratio of ELS production, while 2D, 3A, 3B, 3D, 4D, 5B chromosomes were described which are responsible for plant regeneration efficiency (Zhang and Li 1984; Szakács et al. 1988; Agache et al. 1989; Henry et al. 1994). QTL analyses revealed that the 5A, 5B chromosomes and 1B, 2A, 2B, 5B, 7B chromosomes contribute to the efficiency of ELS production and plant regeneration, respectively (Torp et al. 2001; Nielsen et al. 2015). Lazaridou et al. (2016) proved that the efficiency of in vitro wheat AC (ELS and green plantlets production) decreased in the absence of D genome. Recently, the published in vitro AC protocols are less efficient in tetraploid (AABB) durum wheat (Triticum durum L.) than in hexaploid (AABBDD) common wheat (Triticum aestivum L.).

The above mentioned studies provide a theoretic explanation for the insufficient data of in vitro androgenesis of diploid (AA) recalcitrant Triticum monococcum L. During the last few years, genetic control of somatic tissue culture response was successfully resolved by significant methodological improvements in einkorn (Miroshnichenko et al. 2017, 2018). Therefore, the plant regeneration efficiency can
be increased with further methodology development in the diploid cereal species, einkorn (*Triticum aestivum* L.).

In conclusion, in vitro androgenesis induction of einkorn is reported at first in this study. The long cold pre-treatment (2 weeks, 4 °C) was effective for ELS production. Genotype dependency and genotype x treatment interaction was observed in the experiments. Green and albino plantlets were regenerated from the microspore—derived ELS. The ploidy level of regenerated green plantlet was determined by flow cytometric analyses, the regenerated plantlet was identified as a haploid plant. The in vitro AC is a promising method based on ELS production of einkorn genotypes, but the plant regeneration efficiency requires further improvements for extensive practical application.

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**Data availability** All data generated or analysed during this study are included in this article.

**Declarations**

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

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**References**

Agachie S, Bechelier B, De Buyser J, Henry Y, Snape J (1989) Genetic analyses of anther culture response in wheat using aneuploid, chromosome substitution and translocation lines. Theor Appl Genet 77:7–11. https://doi.org/10.1007/BF00292308

Agil F, Orgec M, Karakas FP, Verma SK, Zencirci N (2021) In vitro mature embryo culture protocol of einkorn (*Triticum monococcum ssp. monococcum*) and bread (*Triticum aestivum* L.) wheat under boron stress. Plant Cell Tiss Org 148:293–304. https://doi.org/10.1007/s11240-021-02186-0

Arzany A, Ashraf M (2017) Cultivated Ancient Wheats (*Triticum* spp.): a potential source of health-beneficial food products. Compr Rev Food Sci Food Saf 16:477–488. https://doi.org/10.1111/1541-4337.12262

Alikina O, Chernobrovkina M, Dolgov S, Miroshnichenko D (2016) Genome editing in wheat microspores and haploid embryos mediated by delivery of ZFN proteins and cell-penetrating peptide complexes. Plant Biotechnol J 18:1307–1316. https://doi.org/10.1111/pbi.13296

Barakat MN, Al-Doss AA, Ghazy AI, Moustafa KA, Elshafei AA, Ahmed EI (2017) Doubled haploid wheat lines with high molecular weight glutenin alleles derived from microspore culture. N Z J Crop Hort Sci 46:198–211. https://doi.org/10.1080/011040671.2017.1368674

Barnabás B (2003) Protocol for producing doubled haploid plants from anther culture of wheat (*Triticum aestivum* L.). In: Maluszynski M, Kashka, Forster BP, Szarejko I (eds) Doubled haploid production in crop plants, a manual. Kluwer Academic Publishers, Dordrecht, pp 65–70

Bilichak A, Sastry-Dent L, SRiram S, Simpson M, Samuel P, Webb S, Jiang F, Eudes F (2020) Genome editing in wheat microspores and haploid embryos. Plant Genet 77:7–11. https://doi.org/10.1007/BF00292308

Chauhan H, Khurana P (2011) Use of haploid technology for development of stable drought tolerant bread wheat (*Triticum aestivum* L.) transgenics. Plant Biotechnol J 9:408–417. https://doi.org/10.1111/j.1467-7652.2010.00561.x

Cistué L, Valles M, Echávarri B, Sanz J, Castillo A (2003) Barley anther culture. In: Maluszynski M, Kashka, Forster BP, Szarejko I (eds) Doubled haploid production in crop plants, a manual. Kluwer Academic Publishers, Dordrecht, pp 29–34

Coelho MB, Scagliusi SMM, Lima MPM, Consoli L, Grando MF (2018) Androgenic response of wheat genotypes resistant to fusariosis. Pesqui Agropecuaria Bras 53:575–582. https://doi.org/10.1590/S0100-204X2018000500006

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

Deaton WR, Metz SG, Armstrong TA, Mascia PN (1987) Genetic analyses of the anther culture response of 3 spring wheat crosses. Theor Appl Genet 74:334–338. https://doi.org/10.1007/BF00274715

Dunwell JM (2010) Haploids in flowering plants: origins and exploitation. In: Maluszynski M, Kashka, Forster BP, Szarejko I (eds) Doubled haploid production in crop plants, a manual. Kluwer Academic Publishers, Dordrecht, pp 29–34

Echávarri B, Cistué L (2016) Enhancement in androgenesis efficiency in barley (* Hordeum vulgare* L.) and bread wheat (*Triticum aestivum* L.) by the addition of dimethyl sulfoxide to the mannitol pretreatment medium. Plant Cell Tiss Org 125:11–22. https://doi.org/10.1007/s11240-015-0923-z

Feuillet C, Travella S, Stein N, Albar L, Nablat A, Keller B (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. Proc Natl Acad Sci USA 100:15253–15258. https://doi.org/10.1073/pnas.2435133100

Galbraith DW, Harkins KR, Maddox JM, Ayres NM, Sharma DP, Firoozabady E (1983) Rapid flow cytometric analysis of the cell cycle in intact plant-tissues. Science 220:1049–1051. https://doi.org/10.1126/science.220.4601.1049

Gáliba G, Kovács G, Sutka J (1986) Substitution analyses of plant regeneration efficiency requires further improvements for extensive practical application.
German A (2011) Anther culture for haploid and doubled haploid production. Plant Cell Tiss Org 104:283–300. https://doi.org/10.1007/s11240-010-9852-z

Henry Y, De Buyser J (1985) Effect of IB/IR translocation on anther culture ability in wheat (Triticum aestivum L.). Plant Cell Rep 4:307–310. https://doi.org/10.1007/BF00269885

Henry Y, Vain P, De Buyser J (1994) Genetic analyses of in vitro plant tissue culture responses and regeneration capacities. Euphytica 79:45–48. https://doi.org/10.1007/BF00023575

Hensel G, Oleszczuk S, Daghigh DES, Jinny J, Melzer M, Kummeln J (2012) Analysis of T-DNA integration and generative segregation in transgenic winter triticale (X Triticosecale Wittmack). BMC Plant Biol 12:171. https://doi.org/10.1186/1471-2229-12-171

Jiang FY, Ryabova D, Diedhiou J, Huc L, Randhawa H, Marillia EF, Henry Y, Vain P, De Buyser J (1994) Genetic analyses of in vitro plant tissue culture response to culture temperature in wheat. Plant Cell Rep 36:1701–1706. https://doi.org/10.1007/s00299-017-2183-3

Jing HC, Komyukhin D, Kanuku K, Orford S, Zlatksa A, Mitrofanova OP, Koebner R, Hammon-Kosack K (2007) Identification of variation in adaptively important traits and genome wide analyses of trait-marker associations in Triticum monococcum. J Exp Bot 58:3749–3764. https://doi.org/10.1093/jxb/erm225

Kumlehn J, Hensel G (2009) Genetic transformation technology in the Triticeae. Breed Sci 59:553–560. https://doi.org/10.1270/jsbbs.59.553

Lantos C, Pauk J (2016) Anther culture as an effective tool in winter wheat (Triticum aestivum L.) breeding. Russ J Genet 52(8):794–801. https://doi.org/10.1134/S102279541608007X

Lantos C, Pauk J (2020) Factors influencing the efficiency of wheat anther culture. Acta Biol Cracov Bot 62:7–15. https://doi.org/10.1270/jabcb.62.7

Lazar MD, Baenziger PS, Schaeffer GW (1984) Combining abilities and heritability of callus formation and plantlet regeneration in wheat (Triticum aestivum L.) anther cultures. Theor Appl Genet 68:131–134. https://doi.org/10.1007/BF00252328

Lazaridou T, Pankou C, Xynias I, Roupakias D (2016) Effect of D genome on wheat anther culture response after cold and mannitol pretreatment. Acta Biol Cracov Bot 58:95–102. https://doi.org/10.1111/pbr.12032

Lantos C, Pauk J (2020) Factors influencing the efficiency of wheat anther culture. Acta Biol Cracov Bot 62:7–15. https://doi.org/10.1270/jabcb.62.7

Lazar MD, Baenziger PS, Schaeffer GW (1984) Combining abilities and heritability of callus formation and plantlet regeneration in wheat (Triticum aestivum L.) anther cultures. Theor Appl Genet 68:131–134. https://doi.org/10.1007/BF00252328

Liu W, Zheng MY, Konzak CF (2001) Improving green plant production via isolated microspore culture in bread wheat (Triticum aestivum L.). Plant Cell Rep 20:821–824. https://doi.org/10.1007/s00299-001-0408-x

Longin CFH, Friedrich H, Würschum T (2016) Back to the future: tapping into ancient grains for food diversity. Trends Plant Sci 21:731–737. https://doi.org/10.1016/j.tplants.2016.05.005

Miroshnichenko D, Ashin D, Pushin A, Dolgov S (2018) Genetic transformation of einkorn (Triticum monococcum spp. monococcum), a diploid cultivated wheat species. BMC Biotechnol 18:68. https://doi.org/10.1186/s12896-018-0477-3

Miroshnichenko D, Chaban I, Chernobrovkina M, Dolgov S (2017) Protocol for efficient regulation of in vitro morphogenesis in einkorn (Triticum monococcum spp. monococcum), a recalcitrant diploid wheat species. PLoS ONE 12:e0173533. https://doi.org/10.1371/journal.pone.017533

Niadian M, Shariatpanahi ME (2020) In vitro-based doubled haploid production: recent improvements. Euphytica 216:69. https://doi.org/10.1007/s10681-020-02699-7

Nielsen NH, Andersen SU, Stougaard J, Jensen A, Backes G, Jahoor L) breeding. Russ J Genet 52(8):794–801. https://doi.org/10.1134/S102279541608007X

Ouyang JW, Zhou SM, Jia SE (1983) The response of anther culture to culture temperature in Triticum aestivum. Theor Appl Genet 66:101–109. https://doi.org/10.1007/BF00265182

Ouyang JW, Jia SE, Zhang C, Chen X, Fen G (1989) A new synthetic medium (W14) for wheat anther culture. Annual Report, pp 91–92. Institute of Genetics, Academia Sinica, Beijing

Park J, Mihály R, Puolimatka M (2003) Protocol of wheat (Triticum aestivum L.) anther culture. In: Maluszynski M, Kashka KJ, Forster BP, Szarejko I (eds) Doubled haploid production in crop plants, a manual. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 59–64

Plamenov D, Belchev I, Spetsov P (2009) Anther culture response of wheat (Triticum durum × T. monococcum spp. aegilopoides) amphidiploid. Cereal Res Commun 37:255–259. https://doi.org/10.1556/CRC.37.2009.2.13

Purnhauser L, Gyulai G (1993) Effect of cooper on shoot and root regeneration in wheat, triticale, rape and tobacco tissue-cultures. Plant Cell Tiss Org 35:131–139. https://doi.org/10.1007/BF00032962

Ren J, Wu P, Trampe B, Tian X, Lübbestetti T, Chen S (2017) Novel technologies in doubled haploid line development. Plant Biotech Nol J 15:1361–1370. https://doi.org/10.1111/pbi.12805

Sanchez-Diaz RA, Castillo AM, Valles MP (2013) Microspore embryogenesis in wheat: new marker genes for early, middle and late stages of embryo development. Plant Reprod 26:287–296. https://doi.org/10.1007/s00497-013-0225-8

Shchukina LV, Pshenichnikova T, Khlestkina EK, Mishaeva S, Kartvelishvili M, Vinter A, Valiev R, Basiev R, Besarab A, Abakumova EI (2013) Back to the future: tapping into ancient grains for food diversity. Trends Plant Sci 21:731–737. https://doi.org/10.1016/j.tplants.2016.05.005

Shchukina LV, Pshenichnikova T, Khlestkina EK, Misheva S, Kartvelishvili M, Vinter A, Valiev R, Basiev A (2013) Chromosomal location and mapping of quantitative trait locus determining technological parameters of grain and flour in strong-flour bread wheat cultivar Saratovskaya 29. Cereal Res Commun 46:628–638. https://doi.org/10.1556/0806.46.2018.047

Shchukina LV, Pshenichnikova T, Khlestkina EK, Misheva S, Kartvelishvili M, Vinter A, Valiev R, Basiev A (2013) Chromosomal location and mapping of quantitative trait locus determining technological parameters of grain and flour in strong-flour bread wheat cultivar Saratovskaya 29. Cereal Res Commun 46:628–638. https://doi.org/10.1556/0806.46.2018.047
Song J, Carver BF, Powers C, Yan L, Klapste J, El-Kassaby YA, Chen C (2017) Practical application of genomic selection in a doubled-haploid wheat breeding program. Mol Breed 37:117. https://doi.org/10.1007/s11032-017-0715-8

Soriano M, Cistué L, Valles MP, Castillo AM (2007) Effects of colchicine on anther and microspore culture of bread wheat (Triticum aestivum L.). Plant Cell Tiss Org 91:225–234. https://doi.org/10.1007/s11240-007-9288-2

Soriano M, Cistué L, Castillo AM (2008) Enhanced induction of microspore embryogenesis after n-butanol treatment in wheat (Triticum aestivum L.) anther culture. Plant Cell Rep 27:805–811. https://doi.org/10.1007/s00299-007-0500-y

Stein N, Feuillet C, Wicker T, Schlagenhaus E, Keller B (2000) Subgenome chromosome walking in wheat: a 450-kb physical contig in Triticum monococcum L. spans the Lr10 resistance locus in hexaploid wheat (Triticum aestivum L.). Proc Natl Acad Sci USA 97:13436–13441. https://doi.org/10.1073/pnas.200361597

Suchowilska E, Wiwart M, Kandler W, Krska R (2012) A comparison of macro- and microelement concentration in the whole grain of four Triticum species. Plant Soil Environ 58:141–147. https://doi.org/10.17221/688/2011-PSE

Testillano PS (2019) Microspore embryogenesis: targeting the determinant factors of stress-induced cell reprogramming for crop improvement. J Exp Bot 70:2965–2978. https://doi.org/10.1093/jxbery/464

Torp AM, Hansen AL, Andersen SB (2001) Chromosomal regions associated with green plant regeneration in wheat (Triticum aestivum L.) anther culture. Euphytica 119:377–387. https://doi.org/10.1023/A:1017554129904

Tuvesson S, Ljungberg A, Johansson N, Karlsson KE, Suijs LW, Posset JP (2000) Large-scale production of wheat and triticale double haploid through the use of a single-anther culture method. Plant Breed 119:455–459. https://doi.org/10.1046/j.1439-0523.2000.00536.x

Tyrka M, Oleszczuk S, Rabiza-Swider J, Wos H, Wedzony M, Zimny J, Ponitka A, Slusarkiewicz-Jarzina A, Metzger RJ, Baenziger PS, Lukaszewski AJ (2018) Populations of doubled haploids for genetic mapping in hexaploid winter triticale. Mol Breed 38:46. https://doi.org/10.1007/s11032-018-0804-3

Uauy C, Distelfeld A, Fahima T, Blechacz A, Dubcovsky J (2006) A NAC gene regulating senescence improves grain protein, zinc and iron content in wheat. Science 314:1298–1301. https://doi.org/10.1126/science.1133649

Wajdzik K, Golebiowska G, Dyda M, Gawronska K, Rapacz M, Wedzony M (2019) The QTL mapping of the important breeding traits in winter triticale (X Triticosecale Wittm.). Cereal Res Commun 47:395–408. https://doi.org/10.1556/08064.2019.24

Wang HM, Enns JL, Brost JM, Orr TD, Ferrie AMR (2019) Improving the efficiency of wheat microspore culture evaluation of pretreatment gradients, and epigenetic chemicals. Plant Cell Tiss Org 139:589–599. https://doi.org/10.1007/s11240-019-01704-5

Weigt D, Kiel A, Siatkowski I, Zyprych-Walczak J, Tomkowiak A, Kwiatek M (2020) Comparison of androgenic response of spring and winter wheat. Plants 9:49. https://doi.org/10.3390/plants9010049

Wessels E, Botes WC (2014) Accelerating resistance breeding in wheat by integrating marker-assisted selection and doubled haploid technology. S Afr J Plant Soil 31:35–43. https://doi.org/10.1080/02571862.2014.903434

Wuerschum T, Tucker MR, Reif JC, Maurer HP (2012) Improved efficiency of doubled haploid generation in hexaploid triticale by in vitro chromosome doubling. BMC Plant Biol 12:109. https://doi.org/10.1186/1471-2229-12-109

Yan L, Loukoianov A, Blechacz A, Tranquilli G, Ramakrishna W, San-Miguel P, B Bennett JL, Echenique V, Dubcovsky J (2004) The wheat VRN2 gene is a flowering repressor down regulated by vernalization. Science 303:1640–1644. https://doi.org/10.1126/science.1094305

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene VRN1. Proc Natl Acad Sci USA 100:6263–6268. https://doi.org/10.1073/pnas.0309739100

Zaharieva M, Monneveux P (2014) Cultivated einkorn wheat (Triticum monococcum L., subsp. monococcum): the long life of a founder crop of agriculture. Genet Resour Crop Evol 61:677–706. https://doi.org/10.1007/s10722-014-0084-7

Zao L, Liu L, Wang J, Guo H, Gu J, Zhao S, Li J, Xie Y (2015) Development of a new wheat germplasm with high anther culture ability by using of gamma-ray irradiation and anther culture. J Sci Food Agric 95:120–125. https://doi.org/10.1002/jsfa.6691

Zhao P, Wang K, Zhang W, Liu HY, Du LP, Hu HR, Ye XG (2017) Comprehensive analyses of differently expressed genes and proteins in albino and green plantlets from a wheat anther culture. Biol Plant 61:255–265. https://doi.org/10.1007/s10535-016-0662-y

Zhang YL, Li DS (1984) Anther culture of monosomics in Triticum aestivum. Hereditas (beijing) 6:7–10

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