Czech Journal of Genetics and Plant Breeding, *Original Paper*

https://doi.org/10.17221/113/2019-CJGPB

Generation of doubled haploid lines from winter wheat (*Triticum aestivum* L.) breeding material using *in vitro* anther culture

Osama Zuhair Kanbar[1,2], *Csaba Lantos*[2], *Paul Karumba Chege*[1,2], *Erzsebet Kiss*[3], *Janos Pauk*[2]*

[1]*Doctoral School of Plant Science, Szent Istvan University, Gödöllő, Hungary*
[2]*Departemant of Biotechnology, Cereal Research Non-Profit Ltd., Szeged, Hungary*
[3]*Genetics, Microbiology and Biotechnology Institute, Szent Istvan University, Gödöllő, Hungary*

**Corresponding author: janos.pauk@gabonakutato.hu*

Citation: Kanbar O.Z., Lantos C., Chege P.K., Kiss E., Pauk J. (2020): Generation of doubled haploid lines from winter wheat (*Triticum aestivum* L.) breeding material using *in vitro* anther culture. Czech J. Genet. Plant Breed.

Abstract: We investigated the anther culture (AC) efficiency of thirteen F_4 combinations of winter wheat (*Triticum aestivum* L.). The genotype dependency was assessed during the induction of the androgenic entities, i.e. embryo-like structures (ELS), regenerated-, green-, albino-, and transplanted plantlets. The number of green plantlets per 100 anthers (GP/100A) varied from 0.36 to 24.74 GP/100A with a mean of 8.31 GP/100A. Albino plantlets (AP) occurred in each combination, ranging from 0.20 to 22.80 AP/100A with an average value of 5.59 AP/100A. Between 25–87.76 doubled haploid (DH) plants per 100 acclimatised plantlets (DH/100ADP), depending on the combination, with a mean of 59.74% were recovered. We have found the highest DH production in the combinations Béres/Midas, Kalász/Tacitus, Béres/Pamier, and Premio/5009. This improves remarkably the choice of basic genetic material in subsequent crossing programmes. These observations emphasise the usability and efficiency of *in vitro* AC in producing a large number of DH lines for breeding and the applied researches of winter wheat. Although albinism was found in each combination, it was mitigated by the *in vitro* AC application.

Keywords: breeding; haploid; *Triticum aestivum* L.; wheat

Abbreviations: AC – anther culture; AP/100A – albino plantlets per 100 anthers; AP/100ELS – albino plantlets per 100 embryo-like structures; AP/100RP – albino plantlets per 100 regenerated plantlets; DH – doubled haploid; DH/100ADP – doubled haploid plants per 100 acclimatised plantlets; ELS – embryo-like structures; GP/100A – green plantlets per 100 anthers; GP/100ELS – green plantlets per 100 embryo-like structures; GP/100RP – green plantlets per 100 regenerated plantlets; RP/100A – regenerated plantlets per 100 anthers; TP/100A – transplanted plantlets per 100 anthers

In cereal crops, the use of the doubled haploid (DH) technology enables genetically homozygous pure lines from heterozygous breeding material to be realised in a single generation (Yan et al. 2017). Improvements and adoption of the technology have rendered it a fast alternative to conventional breeding methods and it has become a requisite tool in the attainment of homogeneity in different research and programmes (Wedzony et al. 2009; Lantos & Pauk 2016; Mahato & Chaudhary 2019). The technology also aids in phenotyping more precisely (Yan et al. 2017) and in genetic studies (Sorrells et al. 2011; Hao et al. 2013; Shi et al. 2019).

Supported by the János Bolyai Research Scholarship of the HAS and by Grants OTKA-K_16-K119835 and TUDFO/51757/2019-ITM. The research Grant: EFOP-3.6.3-VEKOP-16-2017-00008 Project supported the research effectively, too. The latter Project is co-financed by the European Union and the European Social Fund.

https://doi.org/10.17221/113/2019-CJGPB

The methods used in breeding to produce DH lines include wide hybridisation, gynogenesis, and androgenesis (Dunwell 2010). Intergeneric hybridisation, e.g., crossing with maize (*Zea mays* L.) or *Hordeum bulbosum* (Suenaga et al. 1997), anther cultures (AC) (Castillo et al. 2015) and isolated microspore cultures are the methods used for the DH production in wheat (*Triticum aestivum* L.) and other cereals (Lantos & Pauk 2016). Other major crops for which protocols for DH have been applied include barley, triticale, rice, and maize (Dunwell 2010; Niu et al. 2014). Using this plant improvement approach, researchers have already produced registered cultivars (Kush & Virmani 1996) and commercial varieties (Thomas et al. 2003).

In winter wheat, *in vitro* AC has been utilised successfully in many research programmes to release new varieties, for example: Jinghua No-1 (Hu et al. 1986), Florin (De Buyser et al. 1987), GK Délibáb (Pauk et al. 1995), McKenzi (Graf et al. 2003) or AC Andrew (Sadasivaiah et al. 2004). With androgenesis, however, as many researchers have reported for this crop, the DH production is limited by albinism (Islam 2010; Broughton 2011; Lantos et al. 2013). Furthermore, genotype dependency is a major obstacle to wheat AC-induced DH production; the genotypic influence on the AC response limits the effectiveness of the AC method for breeding purposes (Tuvesson et al. 2000; Chen et al. 2011; Kondic-Spika et al. 2011; Dwivedi et al. 2015). In hexaploid wheat, it has been found that winter genotypes are more responsive than spring genotypes (Sharma et al. 2005). Holme et al. (1999) reported that the north-western European wheat genotypes have a lower response compared to their eastern European counterparts. Thus, using a responsive breeding material in crossing was one of the strategies presented by Tuvesson et al. (2000) to increase wheat AC efficiency. The most important aspect of this strategy is that one of the parental genotypes in each cross should produce at least one green plantlet/spike in the AC (Tuvesson et al. 2003).

This study was aimed at producing winter wheat (*T. aestivum* L.) homogeneous lines via *in vitro* androgenesis. The effect of the genotype (combination) factor on the androgenic traits: embryo-like structures (ELS), regenerated plantlets, green plantlets, albino plantlets, transplanted plantlets, was determined. The DH lines generated in the present project will be evaluated in a subsequent programme for drought tolerance and agronomic traits to release germplasms and breeding sources.

MATERIAL AND METHODS

Plant material. Seeds of thirteen F_4 accessions, harvested from the drought tolerance selection trial of thirteen winter wheat F_3 materials (Table 1) at the Cereal Research Non-Profit Ltd. (CR Ltd.), were sown on 5 m^2 plots/accession (450 seed/m^2) at the CR Ltd. in October 2017. Further treatment was carried out according to Lantos et al. (2013).

Collection and treatment of donor tillers. In total, 35–40 donor tillers per genotype with microspores at the vacuolated, early uninucleate stage (determined via an Olympus CK-2 inverted microscope (Olympus, Southern-on-Sea, UK), Figure 1A) were collected, put in Erlenmeyer flasks with tap water, covered with PVC (polyvinyl chloride) bags and kept for 2 weeks at 3–4 °C under continuous dim light (fluorescent light at 200 µmol/m^2/s).

Isolation and incubation of anthers. Selected cold-treated spikes with microspores at the optimal developmental stage were transferred to a flow box, put in 250 mL Erlenmeyer flasks (containing a 200 mL 2% NaOCl solution (w/v) with one drop of Tween-80), covered and placed on a gyratory shaker for 20 min (120 RPM). The spikes were then rinsed three times in sterile distilled water (Millipore Elix 5). In total, 300 anthers were isolated using fine forceps and placed onto a 90 mm plastic Petri dish (Sarstedt, Budapest, Hungary) with 15 mL of a liquid

Table 1. List of the F_4 wheat combinations tested in the anther culture; the crossing combinations were selected in the previous (F_3) generation based on the yield performance and drought tolerance in the different ecological conditions

No.	Code number	Combinations
1	2522	Sel.9/DH150
2	2533	Premio/5009
3	2570	DL41/DH150
4	2572	DL45/DH150
5	2581	Béres/Midas
6	2591	Béres/Pamier
7	2610	Kalász/Tacitus
8	2635	Kolo/Premio
9	2680	Körös/Premio
10	2712	Midas/Csillag//Tacitus/5003
11	2739	DH54/12.189
12	2740	DH54/12.89
13	2744	Kapos/Ködmön

https://doi.org/10.17221/113/2019-CJGPB

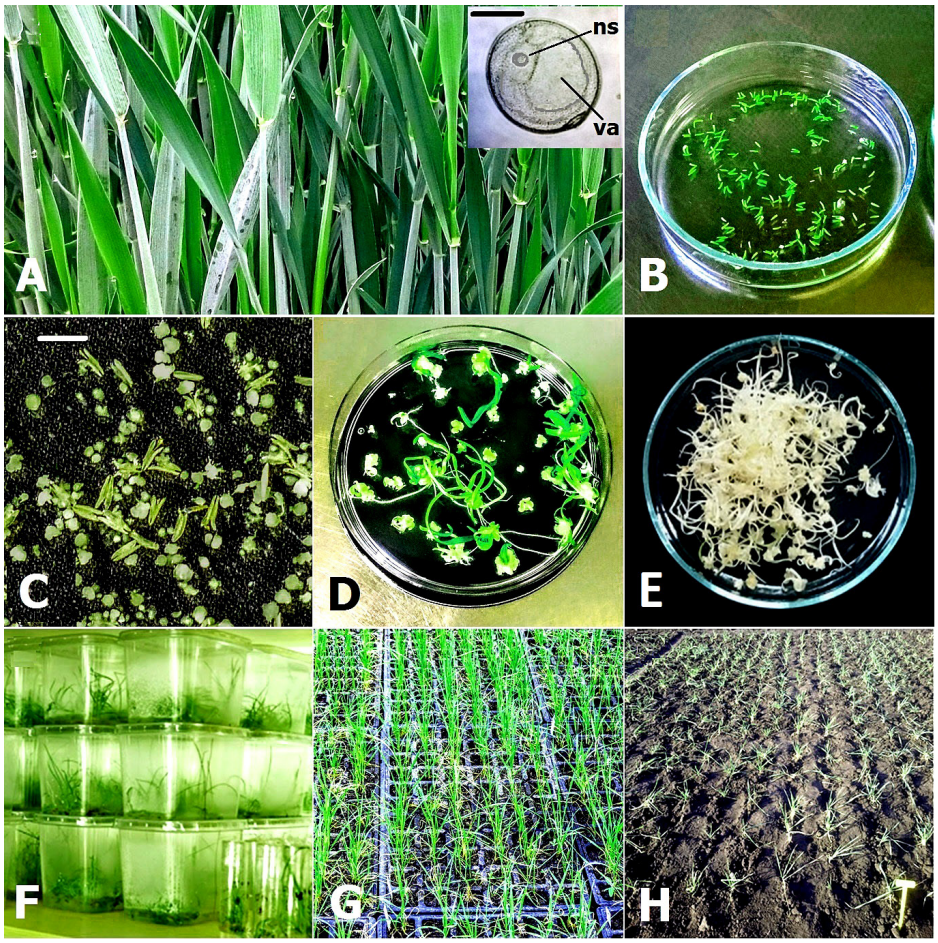

Figure 1. Main stages of the wheat anther culture: donor tillers in the nursery when the microspores are in the uninucleate developmental stage (right-up corner of A; ns – nucleus, va – vacuole) (A); isolated anthers on the surface of the W14mf liquid medium (B); embryo-like structures obtained in the four week-old anther culture (C); green plantlets on the regeneration medium (D); collected and discarded albino plantlets from the plant regeneration (E); well-rooted green plantlets in plastic boxes (F); transplanted plantlets in a glasshouse (G); transplanted plantlets in the field (H)
Bar = 10 μm (A) or 4 mm (C)

W14mf induction medium (Lantos & Pauk 2016, Figure 1B). After heat-shock treatment (32 °C for 36 h in the dark), the cultures were kept at 28 °C in the dark for about 5–8 weeks to induce embryo-like structures. In total, 30–35 cold-pre-treated spikes per genotype were used to prepare 10 replications, with 300 anthers each.

Plantlets regeneration. Approximately 5-weeks after incubation, about 30–35 ELS with a diameter of 1–2 mm (Figure 1C) were transferred onto Petri dishes with 30 mL of a 190-2Cu regeneration medium solidified with 2.8 g/L of Gelrite® (Pauk et al. 2003) and placed in a light room.

After about 2–3 weeks, the green plantlets, 20 to 30 mm long (Figure 1D), were transferred into 1000 mL plastic boxes with a solid regeneration medium (15 plantlets/box) or individually – when the regenerates could be recovered – into 50 mL glass tubes with the same medium, and kept in a growth room (24 °C, 16/8 h light/dark photoperiod, fluorescent light at 200 μmol/m^2/s) for the entire plantlets regeneration (Figure 1F). The albino plantlets were counted and discarded (Figure 1E).

Acclimatisation of plantlets and harvesting of DH seeds. After about 4–5 weeks, the well-rooted plantlets were transplanted to plastic tracks (Figure 1G) filled with peat and sandy soil mixture (1 : 1) and grown in a glasshouse. For plant acclimatisation, the plantlets were initially kept under a PVC cover for 3–5 days at 15–20 °C. After 2–3 weeks, the plants

https://doi.org/10.17221/113/2019-CJGPB

Table 2. Statistical analysis of the androgenic parameters per 100 anthers for thirteen F_4 wheat combinations by the one-way ANOVA

Source of variance	*df*	Mean squares				
		No. of ELS/100A	No. of RP/100A	No. of GP/100A	No. of AP/100A	No. of TP/100A
Combination	12	5349***	1329.8***	654***	440.1***	180.96***
Error	111	272	58.7	31.1	16.4	13.85

***The values significantly differ at $P < 0.001$; ELS/100A – embryo-like structures/100 anthers; RP/100A – regenerated plantlets per 100 anthers; GP/100A – green plantlets per 100 anthers; AP/100A – albino plantlets per 100 anthers; TP/100A – transplanted plantlets per 100 anthers

were transferred to a cool chamber (16/8 h light/dark, 8–12 °C) for an additional 1–3 months.

In October, the plantlets were transplanted from the cool chamber to the field (Figure 1H). At the end of the growing season the following year, all of the at least partially fertile spikes were manually harvested; the plants with only sterile spikes were counted and discarded. Before grain thrashing, the plants were sorted into two groups based on the type of spike fertility: fully (100% seed set in the spike) and partially (< 100%) fertile.

Statistical analysis. To evaluate the effect of the genotype, the data of the androgenic parameters (number of ELS, regenerated-, green-, albino-, and transplanted plantlets) was analysed by a one-way ANOVA (analysis of variance) using R software (Ver. 3.6.1., R Core Team, 2019). The pairwise comparisons of the means were computed as well.

RESULTS

The statistical analysis revealed that the effect of the genotype was significant for all the tested androgenic parameters (the number of embryo-like structures, regenerated plantlets, green and albino plantlets, and the well-rooted transplanted plantlets) at $P < 0.001$ (Table 2).

Evaluation of androgenic traits. Table 3 summarises the significant differences among the genotypes for all the examined traits. The quantity of the

Table 3. Androgenic response per 100 anthers for thirteen F_4 wheat combinations in the anther culture

Code of combinations	No. of ELS/100A	No. of RP/100A	No. of GP/100A	No. of AP/100A	No. of TP/100A
2522	20.30^{ce}	9.60^{d}	5.20^{def}	4.40^{de}	4.33^{cdf}
2533	44.07^{bc}	26.30^{ab}	24.74^{a}	1.56^{e}	9.67^{abc}
2570	34.38^{cd}	12.52^{cd}	6.71^{cf}	5.81^{de}	5.24^{bef}
2572	25.50^{ce}	10.50^{d}	9.00^{ce}	1.50^{e}	6.37^{bde}
2581	39.23^{c}	23.80^{bc}	22.13^{ab}	1.67^{e}	12.60^{a}
2591	74.53^{a}	30.86^{ab}	15.93^{bc}	14.93^{c}	11.43^{ab}
2610	71.03^{a}	36.26^{a}	13.46^{cd}	22.80^{b}	8.83^{abd}
2635	6.00^{e}	0.56^{d}	0.36^{f}	0.20^{e}	0.33^{f}
2680	39.66^{c}	11.97^{d}	1.80^{ef}	10.17^{cd}	1.00^{ef}
2712	65.33^{ab}	6.60^{d}	3.70^{ef}	2.90^{e}	3.36^{df}
2739	13.10^{de}	3.83^{d}	2.37^{ef}	1.46^{e}	1.50^{ef}
2740	24.46^{ce}	4.29^{d}	1.50^{ef}	2.79^{e}	1.33^{ef}
2744	6.43^{e}	2.50^{d}	0.90^{ef}	1.60^{e}	0.73^{ef}
Mean	35.84	13.90	8.31	5.59	5.16
$LSD_{0.05}$	15.50	7.20	5.24	3.81	3.50

The values followed by the same letters within a column are not significantly different at $P = 0.05$ probability levels as determined by the pairwise comparison of the means test (Tukey Contrasts); ELS/100A – embryo-like structures per 100 anthers; RP/100A – regenerated plantlets per 100 anthers; GP/100A – green plantlets per 100 anthers; AP/100A – albino plantlets per 100 anthers; TP/100A – transplanted plantlets per 100 anthers

https://doi.org/10.17221/113/2019-CJGPB

Table 4. Statistical analysis of the androgenic parameters, green plantlets (GP) and albino plantlets (AP) per 100 embryo-like structures (100ELS) and per 100 regenerated plantlets (100RP) for thirteen F_4 wheat combinations by the one-way ANOVA

Source of variance	*df*	Mean squares		*df*	Mean squares	
		No. of GP/100ELS	No. of AP/100ELS		No. of GP/100RP	No. of AP/100RP
Combination	12	2850.9***	1137.7***	12	5343***	5343***
Error	111	93.6	96.9	108	330	330

***The values significantly differ at $P < 0.001$

embryo-like structures per 100 anthers (ELS/100A) ranged from 6.00 to 74.53, depending on the combination (genotype), while the overall mean was 35.84 ELS/100A (Table 3).

The values of the regenerated plantlets per 100 anthers (RP/100A) varied from 0.56 in the Kolo/Premio combination to 36.26 in the Kalász/Tacitus one with a mean of 13.90 RP/100A (Table 3). Out of the RP/100A, the number of green plantlets per 100 anthers (GP/100A) ranged from 0.36 to 24.74. The combinations the Premio/5009, Béres/Midas, and Béres/Pamier combinations showed high values of GP/100A (24.74, 22.13, and 15.93, respectively) while the Kolo/Premio, Kapos/Ködmön and DH54/12.89 combinations had the lowest values (0.36, 0.90 and 1.50, respectively). The mean was 8.31 GP/100A (Table 3).

Albino plantlets (AP) were observed in each combination. However, the values per 100 anthers (A) varied from 0.20 to 22.80. The mean value was 5.59 AP/100A (Table 3). Table 3 shows the range of the transplanted plantlets per 100 anthers (TP/100A), varying from 0.33 to 12.60. The combinations with the highest values were Béres/Midas, Béres/Pamier and Premio/5009 (12.60, 11.43 and 9.67 TP/100A, respectively) while the overall mean was 5.16.

The effect of the combination on the green plantlets per 100 ELS (GP/100ELS), albino plantlets per 100 ELS (AP/100ELS), green plantlets per 100 regenerated plantlets (GP/100RP), and albino plantlets per 100 regenerated plantlets (AP/100RP) was significant at $P < 0.001$ (Table 4). The values of GP/100ELS varied from 4.87 in DH54/12.89 to 55.98 in the Béres/Midas combination while the overall mean was 22.66 GP/100ELS (Table 5). An average of 15.32 AP/100ELS ranged from 3.63 in the Kolo/Premio combination to 34.74 in the Kalász/Tacitus combination (Table 5).

Table 5. The androgenic response of the green plantlets (GP) and albino plantlets (AP) per 100 embryo-like (100ELS) structures and per 100 regenerated plantlets (100RP) for thirteen F_4 wheat combinations in the anther culture

Combinations	No. of GP/100ELS	No. of AP/100ELS	No. of GP/100RP	No. of AP/100RP
2522	26.12^{bc}	27.11^{ab}	49.32^{ce}	50.68^{cd}
2533	55.91^{a}	4.14^{f}	93.25^{a}	6.75^{f}
2570	21.43^{bdf}	17.29^{bdf}	$59.20b^{ce}$	40.80^{cde}
2572	35.64^{b}	5.74^{ef}	84.31^{ab}	15.69^{ef}
2581	55.98^{a}	4.57^{f}	92.35^{a}	7.65^{f}
2591	21.24^{bd}	19.73^{bcde}	50.22^{ce}	49.78^{cd}
2610	19.20^{cde}	34.74^{a}	35.48^{def}	64.52^{abc}
2635	7.38^{de}	3.63^{f}	69.73^{ac}	30.27^{df}
2680	5.19^{e}	25.83^{acd}	16.24^{f}	83.76^{a}
2712	6.11^{ef}	5.25^{ef}	54.03^{ce}	45.97^{cd}
2739	17.14^{cde}	11.88^{cf}	58.05^{bcd}	41.95^{bde}
2740	4.87^{ef}	11.04^{df}	28.73^{ef}	71.27^{ac}
2744	17.76^{cde}	26.82^{ad}	41.25^{cf}	58.75^{ad}
Mean	22.66	15.32	56.07	43.93
$LSD_{0.05}$	9.09	9.25	17.07	17.07

The values followed by the same letters within a column are not significantly different at $P = 0.05$ probability levels as determined by the pairwise comparison of the means test (Tukey Contrasts)

https://doi.org/10.17221/113/2019-CJGPB

Moreover, the number of GP/100RP differed from 16.24 to 93.25 depending on the combination, with a mean of 56.07. The combinations; Béres/Midas, Premio/5009 and DL45/DH150 had the highest values of GP/100ELS (55.98, 55.91 and 35.64, respectively), and also achieved the highest values of GP/100RP, but in a different order: Premio/5009, Béres/Midas and DL45/DH150 – 93.25, 92.35 and 84.31 GP/100RP, respectively (Table 5). The values of AP/100RP ranged between 6.75 in the Premio/5009 combination and 83.76 in the Körös/Premio combination, with an overall mean of 43.93 (Table 5).

Production of DH lines. In total, 1 545 acclimatised plantlets (ADP) were obtained (Table 6). The highest numbers were obtained in the combinations: Béres/Midas, Béres/Pamier, Kalász/Tacitus, and Premio/5009 (301, 270, 239 and 194, respectively). There was a total of 923 spontaneous doubled haploids recovered in the nursery with a mean of 59.74/100 acclimatised plantlets. The rate of DH/100ADP varied from 25 to 87.76% across the combinations. The highest values of the DH plants were present in the combinations Béres/Midas, Kalász/Tacitus, Béres/Pamier, and Premio/5009 – 191, 183, 127, and 120, respectively (Table 6).

DISCUSSION

Recently, anther culture has been widely adopted in breeding and applied research, rendering it a highly-efficient requisite tool with lower costs than alternative technologies in cereal improvements for the development of homogeneity (Castillo et al. 2015). Many researchers have concluded that albinism and the genotype dependency are limiting factors of AC-induced androgenesis in wheat (Jauhar et al. 2009; Chen et al. 2011; Islam & Tuteja 2012; Niu et al. 2014; Dwivedi et al. 2015). This paper shows that these phenomena are not essential hindering factors, because, on average, a similar quantity of albino and green plantlets was regenerated and, subsequently, doubled haploid lines were produced in all the genotypes. Only the green plants were advanced in the breeding programme.

In the studies by researchers: Kondic-Spika et al. (2008); Lantos et al. (2013); and Lantos and Pauk (2016), no unresponsive wheat plant material was observed (without ELS or green plantlets production), but the inverse was observed in the results of Holme et al. (1999); Tuvesson et al. (2000); Broughton (2008); and El-Hennawy et al. (2011). Holme et al.

Table 6. Production of the spontaneous DH plants from thirteen F_4 wheat breeding combinations after induction of the *in vitro* androgenesis via the anther culture

Code of combinations	Total No. of GP	Total No. and percentage of TP		Total No. and percentage of ADP		Number and percentage of DH plants					
						fully fertile		partially fertile		total No. and percentage	
		No.	%	No.	%	No.	%	No.	%	No.	%
2522	156	130	83.33	83	63.85	15	18.07	28	33.73	43	51.81
2533	668	261	39.07	194	74.33	15	7.73	105	54.12	120	61.86
2570	144	110	76.39	88	80.00	15	17.05	22	25.00	37	42.05
2572	270	191	70.74	146	76.44	40	27.40	49	33.56	89	60.96
2581	664	378	56.93	301	79.63	84	27.91	107	35.55	191	63.46
2591	478	343	71.76	270	78.72	87	32.22	40	14.81	127	47.04
2610	404	265	65.59	239	90.19	51	21.34	132	55.23	183	76.57
2635	11	10	90.91	8	80.00	2	25.00	0	0.00	2	25.00
2680	54	30	55.56	29	96.67	5	17.24	5	17.24	10	34.48
2712	111	101	90.99	99	98.02	18	18.18	28	28.28	46	46.46
2739	71	53	74.65	49	92.45	12	24.49	31	63.27	43	87.76
2740	36	32	88.89	31	96.88	8	25.81	19	61.29	27	87.10
2744	27	22	81.48	8	36.36	5	62.50	0	0.00	5	62.50
Total No.	3094	1926	–	1545	–	357	–	566	–	923	–
Overall mean	–	–	61.99	–	80.08	–	23.11	–	36.63	–	59.74

DH – doubled haploid; GP – green plantlets; TP – transplanted plantlets; ADP – acclimatised plantlets

https://doi.org/10.17221/113/2019-CJGPB

(1999) reported that eastern European wheat was more responsive compared with the north-eastern European genotypes. In the current study, the plant material response to *in vitro* AC induction may not have been affected by the geographical origin (eastern and western Europe) since each genotype produced DH lines.

The values of the embryo-like structures per 100 anthers (ELS/100A), ranging from 6.00 to 74.53, and the maximum value was relatively higher than those obtained by previous researches: 53% (Kim & Baenziger 2005); 52% (Khiabani et al. 2008); 18% (El-Hennawy et al. 2011); and 42% (Grauda et al. 2014). The highest ELS/100A exceeding 100% were found in these studies: 119% (Kondic-Spika et al. 2008); and 169.40%, 190.40% in 2010, 2011 (Lantos et al. 2013).

The rate of green plantlets per 100 anthers (GP/100A) obtained in this study was 8.31 which was a bit higher compared to the values ranging from 0.40 to 5.80 GP/100A obtained from different earlier winter wheat breeding programmes (Masojc et al. 1993; Holme et al. 1999; Tuvesson et al. 2000; Kondic-Spika et al. 2008; El-Hennawy et al. 2011; Lantos et al. 2013; Grauda et al. 2014).

The maximum GP/100A value was 24.74. Some authors have reported higher than 100 GP/100A in some high-responding genotypes (Broughton 2011; Lantos et al. 2013; Castillo et al. 2015); others report the maximum values of the GP/100A, just slightly higher than those obtained in this study (Trottier et al. 1993; Navarro-Alvarez et al. 1994; Lantos et al. 2013) while others report less than the current maximum value (Kim & Baenziger 2005; Khiabani et al. 2008; Kondic-Spika et al. 2008; El-Hennawy et al. 2011; Grauda et al. 2014). The strategy of Tuvesson et al. (2000, 2003), involved inserting responsive genotypes in crossing programmes to increase the AC efficiency, and it resulted in a reduction of the time to obtain new DH lines. This highlights the effectiveness of the AC method in practical breeding programmes especially when both green plantlets and DH lines are produced from every investigated genotype.

The number of albino plantlets per 100 anthers (AP/100A) in this study ranged from 0.20 to 22.80 with the overall mean of 5.59, which was low compared to the means in earlier studies (Broughton 2011; El-Hennawy et al. 2011; Lantos et al. 2013; Lantos & Pauk 2016). Ten wheat combinations among the thirteen obtained low values of the AP/100A which were not significantly different ($P < 0.05$) (0.20–5.81%). These results are in contrast to the findings by Wedzony et al. (2009) and Jauhar et al. (2009), who inferred that albinism hindered the androgenic DH production in some genotypes. The genotype dependency was also shown, as DH plants were obtained across the different genotypes. Similar to the results provided by Tuvesson et al. (2000); Kondic-Spika et al. (2011); Lantos and Pauk (2016), the effect of genotype was significant for all the investigated androgenic traits with essential numbers being recorded for the poorest of the genotypes.

In comparison with the rate of the spontaneous DH plants per 100 acclimatised plantlets (DH/100ADP) in the previous experiments, the total DH/100ADP lines rate in this study was higher than those obtained by Kim and Baenziger (2005), Kondic-Spika et al. (2008), Lantos et al. (2013), and Lantos and Pauk (2016), who reported 49%, 47.90%, 35%, and 32.72%, respectively. Spontaneous DH wheat lines that ranged from 5 to 30% were reported by Ziegler et al. (1990); Masojc et al. (1993); and Navarro-Alvarez et al. (1994). In this trial, three combinations had higher DH/100ADP values (76.57%, 87.10%, and 87.76%) than the average, pointing to the parameter as a remarkable tool for the selection of responsive genotypes in order to integrate them into cross-breeding programmes, hence realising increased AC efficiency.

CONCLUSION

This study revealed the importance of *in vitro* haploid induction via androgenesis in a winter wheat breeding programme; each genotype produced green plantlets and doubled haploid plants in essential numbers. The phenomenon of albinism was obtained in each genotype. Although the fluctuation of the anther culture was observed, the genotype dependency was not the inhibiting factor. The combinations: Béres/Midas, Kalász/Tacitus, Béres/Pamier, and Premio/5009 had the highest values of the DH production. Their corresponding DH lines may be effective basic genetic material in crossing programmes for the better improvement in the AC-induced DH plant numbers in future experiments. The generated DH lines (923 DH lines) will be used for further drought-tolerance experiments, to release improved drought-tolerant candidates.

Acknowledgements. The first author is grateful to Stipendium Hungaricum, the scholarship programme of the Hungarian government for supporting this work. Thanks goes to E. Buza for the grammatical corrections.

https://doi.org/10.17221/113/2019-CJGPB

REFERENCES

Broughton S. (2011): The application of *n*-butanol improves embryo and green plant production in anther culture of Australian wheat (*Triticum aestivum* L.) genotypes. Crop and Pasture Science, 62: 813–822.

Broughton S. (2008): Ovary co-culture improves embryo and green plant production in anther culture of Australian spring wheat (*Triticum aestivum* L.). Plant Cell, Tissue and Organ Culture, 95: 185–195.

Castillo A.M., Sánchez-Díaz R.A., Vallés M.P. (2015): Effect of ovary induction on bread wheat anther culture: ovary genotype and developmental stage, and candidate gene association. Frontiers in Plant Science, 6: 402.

Chen J.F., Cui L., Malik A.A., Mbira K.G. (2011): *In vitro* haploid and dihaploid production via unfertilized ovule culture. Plant Cell, Tissue and Organ Culture, 104: 311–319.

De Buyser J., Henry Y., Lonnet P., Herzog R., Hespel A. (1987): Florin – a doubled haploid wheat variety developed by the anther culture method. Plant Breeding, 98: 53–56.

Dunwell J.M. (2010): Haploids in flowering plants: origins and exploitation. Plant Biotechnology Journal, 8: 377–424.

Dwivedi S.L., Britt A.B., Tripathi L., Sharma S., Upadhyaya H.D., Ortiz R. (2015): Haploids: constraints and opportunities in plant breeding. Biotechnology Advances, 33: 812–829.

El-Hennawy M.A., Abdalla A.F., Shafey S.A., Al-Ashkar I.M. (2011): Production of doubled haploid wheat lines (*Triticum aestivum* L.) using anther culture technique. Annals of Agricultural Sciences, 56: 63–72.

Graf R.J., Hucl P., Orshinsky B.R., Kartha K.K. (2003): "McKenzi" hard red spring wheat. Canadian Journal of Plant Science, 83: 565–569.

Grauda D., Miķelsone A., Ļisina N., Žagata K., Ornicāns R., Fokina O., Lapiòa L., Rashal I. (2014): Anther culture effectiveness in producing doubled haploids of cereals. Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences, 68: 142–147.

Hao M., Zhang L., Luo J., Yuan Z., Yan Z., Zhang B. (2013): The genetic study utility of a hexaploid wheat DH population with non-recombinant A- and B-genomes. SpringerPlus, 2: 131.

Holme I.B., Olesen A., Hansen N.J.P., Andersen S.B. (1999): Anther and isolated microspore culture response of wheat lines from northwestern and eastern Europe. Plant Breeding, 118: 111–117.

Hu D.F., Yuan Z.D., Tang Y.L., Liu J.P. (1986): "Jinghua No-1" a winter-wheat variety derived from pollen sporophyte. Science in China Series B – Chemistry, Biological, Agricultural, Medical and Earth Sciences, 29: 733–745.

Islam S.M.S. (2010): Effect of embryoids age, size and shape for improvement of regeneration efficiency from microspore-derived embryos in wheat (*Triticum aestivum* L.). Plant Omics, 3: 149–153.

Islam S.M.S., Tuteja N. (2012): Enhancement of androgenesis by abiotic stress and other pretreatments in major crop species. Plant Science, 182: 134–144.

Jauhar P.P., Xu S.S., Baenziger P.S. (2009): Haploidy in cultivated wheats: induction and utility in basic and applied research. Crop Science, 49: 737–755.

Khiabani B.N., Vedadi C., Rahmani E., Shalmani M.A.M. (2008): Response of some Iranian wheat genotypes to anther culture system. Indian Journal of Biotechnology, 7: 531–535.

Kim K.M., Baenziger P.S. (2005): A simple wheat haploid and doubled haploid production system using anther culture. *In Vitro* Cellular and Developmental Biology-Plant, 41: 22–27.

Kondic-Spika A.Đ., Kobiljski B.Đ., Hristov N.S. (2008): Efficiency of anther culture technique in the production of wheat double haploids. Zbornik Matice Srpske za Prirodne Nauke, 2008: 35–40.

Kondic-Spika A., Vukosavljev M., Kobiljski B., Hristov N. (2011): Relationships among androgenetic components in wheat and their responses to the environment. Journal of Biological Research, 16: 217–223.

Kush G.S., Virmani S.S. (1996): Haploids in plant breeding. In: Mohan Jain S., Sopory S.K., Veilleux R.E. (eds.): *In vitro* Production in Higher Plants. Dordrecht/Boston/London, Kluwer Academic Publishers: 11–34.

Lantos C., Pauk J. (2016): Anther culture as an effective tool in winter wheat (*Triticum aestivum* L.) breeding. Russian Journal of Genetics, 52: 794–801.

Lantos C., Weyen J., Orsini J.M., Gnad H., Schlieter B., Lein V., Kontoeski S., Jacobi A., Mihaly R., Broughton S., Pauk J. (2013): Efficient application of *in vitro* anther culture for different European winter wheat (*Triticum aestivum* L.) breeding programmes. Plant Breeding, 132: 149–154.

Mahato A., Chaudhary H.K. (2019): Auxin induced haploid induction in wide crosses of durum wheat. Cereal Research Communications, 47: 552–565.

Masojc P.O., Lukow M., McKenzie R.I.H., Howes N.K. (1993): Responsiveness to anther culture in cultivars and F_1 crosses of spring wheat. Canadian Journal of Plant Science, 73: 777–783.

Navarro-Alvarez W., Baenziger P.S., Eskridge K.M., Hugo M., Gustafson V.D. (1994): Addition of colchicine to wheat anther culture media to increase doubled haploid plant production. Plant Breeding, 112: 192–198.

Niu Z., Jiang A., Abu Hammad W., Oladzadabbasabadi A., Xu S.S., Mergoum M., Elias E.M. (2014): Review of dou-

https://doi.org/10.17221/113/2019-CJGPB

bled haploid production in durum and common wheat through wheat × maize hybridization. Plant Breeding, 133: 313–320.

Pauk J., Kertész Z., Beke B., Bóna L., Csősz M., Matuz J. (1995): New winter wheat variety – "GK Délibáb" developed via combining conventional breeding and *in vitro* androgenesis. Cereal Research Communications, 23: 251–256.

Pauk J., Mihály R., Puolimatka M. (2003): Protocol of wheat (*Triticum aestivum* L.) anther culture. In: Maluszynski M., Kasha K.J., Forster B.P., Szarejko I. (eds.): Doubled Haploid Production in Crop Plants, a Manual. Dordrecht, Springer: 59–64.

R Core Team (2019): R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria.

Sadasivaiah R.S., Perkovic S.M., Pearson C., Postman B., Beres B.L. (2004): Registration of "AC Andrew" wheat. Crop Science, 44: 696–697.

Sharma S., Sethi G.S., Chaudhary H.K. (2005): Influence of winter and spring wheat genetic backgrounds on haploid induction parameters and trait correlations in the wheat × maize system. Euphytica, 144: 199–205.

Shi Y.G., Lian Y., Shi H.W., Wang S.G., Fan H., Sun D.Z., Jing R.L. (2019): Dynamic analysis of QTLs for green leaf area duration and green leaf number of main stem in wheat. Cereal Research Communications, 47: 250–263.

Sorrells M.E., Gustafson J.P., Somers D., Chao S., Benscher D., Guedira-Brown G., Huttner E., Kilian A., McGuire P.E., Ross K., Tanaka J., Wenzl P., Williams K., Qualset C.O. (2011): Reconstruction of the synthetic W7984 × Opata M85 wheat reference population. Genome, 54: 1–8.

Suenaga K., Morshedi A.R., Darvey N.L. (1997): Haploid production of Australian wheat (*Triticum aestivum* L.) cultivars through wheat × maize (*Zea mays* L.) crosses. Australian Journal of Agricultural Research, 48: 1207–1212.

Thomas W.T.B., Forster B.P., Gertsson B. (2003): Doubled haploids in breeding. In: Maluszynski M., Kasha K.J., Forster B.P., Szarejko I. (eds.): Doubled Haploid Production in Crop Plants: A Manual. Norwell, Kluwer Academic Publishers: 337–350.

Trottier M.C., Collin J., Comeau A. (1993): Comparison of media for their aptitude in wheat anther culture. Plant Cell, Tissue and Organ Culture, 35: 59–67.

Tuvesson S., Ljungberg A., Johansson N., Karlsson K.E., Suijs L.W., Josset J.P. (2000): Large-scale production of wheat and triticale double haploids through the use of a single-anther culture method. Plant Breeding, 119: 455–459.

Tuvesson S.A., Von Post R., Ljungberg A. (2003): Wheat anther culture. In: Maluszynski M., Kasha K.J., Forster B.P., Szarejko I. (eds.): Doubled Haploid Production in Crop Plants, a Manual. Dordrecht/Boston/London, Kluwer Academic Publishers: 71–76.

Wędzony M., Forster B.P., Żur I., Golemiec E., Szechyńska-Hebda M., Dubas E., Gotębiowska G. (2009): Progress in doubled haploid technology in higher plants. In: Touraev A., Forster B.P., Jain, S.M. (eds.): Advances in Haploid Production in Higher Plants. Dordrecht, Springer: 1–33.

Yan G., Liu H., Wang H., Lu Z., Wang Y., Mullan D., Hamblin J., Liu C. (2017): Accelerated generation of selfed pure line plants for gene identification and crop breeding. Frontiers in Plant Science, 8: 1786.

Ziegler G., Dressler K., Hess D. (1990): Investigations on the anther culturability of four German spring wheat cultivars and the influence of light on regeneration of green vs. albino plants. Plant Breeding, 105: 40–46.

Received: November 22, 2019
Accepted: June 19, 2020
Published online: September 4, 2020