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Anther culture response in F_1 hybrids of winter wheat (*Triticum aestivum* L.)

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Abstract. The effect of cold pretreatment of spikes on somatic embryo induction and anther culture response of 25 F_1 winter wheat hybrids was investigated. The efficiency of androgenic embryos was the highest when spikes were incubated at 4°C for 6-9 days. A total of 2242 (73.0%) green and 885 (27.0%) albino plants were obtained from 9900 cultured anthers. Anther culture response in wheat was found to be markedly affected by the genotype of donor plants. The percentage of green plants varied from 0 to 115.7%. A great majority of anther-derived regenerants were haploids (82.35%), while the remaining plants were spontaneous diploids (13.73%) and aneuploids (3.92%).

Key words: anther culture, cold treatment, haploid, hybrids, plant regeneration, somatic embryos, *Triticum aestivum*, winter wheat.

Introduction

The application of the anther culture method for haploid production of wheat plants has been a well-known practice since the seventies (CHU et al. 1973, OUYANG et al. 1973, PICARD, de BUYSER 1973). Haploids can be used after multiplication of their chromosome number which results in a double haploid (DH) state of cells. Anther cultures of wheat became especially attractive in the mid-1980's due to a possibility to reduce breeding cycles (SNAPE 1989). Special breeding programmes of wheat which included androgenic haploids resulted in release of new varieties in some countries: 28 in China, 2 in Hungary and 1 in France (HU et al. 1983, 1985, de BUYSER et al. 1987, HENRY, de BUYSER 1994, PAUK et al. 1995). In relation to promising results

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of numerous successful researches on induced androgenesis and regeneration of haploid plants the number of obtained new varieties is very small. It is assumed that there are several reasons responsible for that, such as genotype, physiology of the anther-donor plants and composition of media used in anther culture, as well as growing conditions of haploids and DH lines (BULLOCK et al. 1982, OUYANG et al. 1983, HENRY, de BUYSER 1990, LAZAR et al. 1990, MASOJĆ et al. 1993, ABD EL-MAKSOUD, BEDO 1993, ORSHINSKY, SADASIVAIAH 1994, EKIZ, KONZAK 1994).

The aim of the present work was to study the effect of cold treatment of spikes on androgenic response and production of haploids from different wheat genotypes.

Material and methods

Anther-donor plants of *Triticum aestivum* (L.) were 25 F_1 hybrids (see Table 2) obtained from the Plant Breeding Station Szelejewo (Poland). The donor plants were grown under field conditions. Tillers were harvested when most microspores were at the mid to late uninucleate stage of development, as assessed by acetocarmine staining of selected squashed anthers. The tillers placed in fresh solution of the mineral salt medium N6 (CHU et al. 1975) were cold pretreated at 4°C in darkness for 2-18 days. After that the spikes were surface-sterilized with 5% calcium hypochlorite for 5 minutes and then washed three times in distilled water. Then the anthers of the two basal florets of spikelet were excised and inoculated onto basal medium potato-2 (CHUANG et al. 1978) with increased 2,4-D concentration up to 2 mg l^{-1} and containing agarose 6 g l^{-1} (SERVA high EEO).The anthers (200 per one 6-cm diameter Petri dish) were incubated in the dark at 30°C. After 3-4 weeks, the somatic embryos developed from microspores were kept in light of 4000 luxes for 16 hrs a day on (R) regeneration medium (KNO_3 – 1000 mg l^{-1}, $(NH_4)_2SO_4$ – 200 mg l^{-1}, KH_2PO_4 – 300 mg l^{-1}, $Ca(NO_3)_2 \times 4H_2O$ – 100 mg l^{-1}, $MgSO_4 \times 7H_2O$ – 200 mg l^{-1}, KCl – 40 mg l^{-1}, KJ – 0.5 mg l^{-1}, $MnSO_4 \times 4H_2O$ – 8.0 mg l^{-1}, H_3BO_3 – 3.0 mg l^{-1}, $ZnSO_4 \times 7H_2O$ – 3.0 mg l^{-1}, $FeSO_4 \times 7H_2O$ – 27.8 mg l^{-1}, $Na_2EDTA \times 2H_2O$ – 37.8 mg l^{-1}, pyridoxine(HCl) – 0.5 mg l^{-1}, nicotic acid – 0.5 mg l^{-1}, thiamine(HCl) – 1.0 mg l^{-1}, myo-inositol – 100 mg l^{-1}, glycine – 2.0 mg l^{-1}, sucrose – 30 g l^{-1}, NAA – 0.5 mg l^{-1}, kinetin – 0.5 mg l^{-1}, agarose – 6 g l^{-1}, pH 6.0). Green plantlets were then transferred into tubes containing R medium and subsequently transplanted into pots. One month after, the plants were vernalized at 4°C for 8 weeks.

The ploidy level of 51 green anther-derived plants was determined in the root tip cells. The roots were fixed in AA solution (3:1 ethyl absolute alcohol : glacial acetic acid), hydrolized in 1 N HCl at 60° C for 7-10 min, stained by the Feulgen method and squashed.

Results

Somatic embryos derived from microspores, emerged from broken anthers after three to four weeks of culture (Fig. 1). At this stage most of somatic embryos were rounded bodies showing similarity to globular zygotic embryos. They distinctly differed from callus structure and were not connected with anther wall. It was found that the frequency of somatic embryo production depends on the length of cold pretreatment of spikes placed in mineral salt solution. The frequency of somatic embryos induction from the anthers of Alba × EGRQ hybrid was the highest when spikes were cold pretreated at 4°C for 6-9 days which is seen from Table 1 below.

Table 1. Effects of cold pretreatment for 2 - 18 days on anther culture responses in the wheat hybrid Alba × EGRQ

Pretreatment (days)	Number of anthers	Somatic embryos	
		No.	(%)
2	800	31	(3.9)
4	800	19	(2.4)
6	1000	89	(8.9)
7	800	148	(18.5)
8	1400	232	(16.6)
9	400	89	(22.3)
11	800	70	(8.8)
18	200	12	(6.0)
Total	6200	690	(11.1)

Therefore, in the experiments with 25 F_1 wheat hybrids the spikes were pretreated at 4°C for 6-9 days.

Table 2. Green and albino plants obtained after anther culture of 25 wheat hybrids

No.	Hybrid	Number of anthers	Plants		
			Green		Albino
			No.	(%)	No.
1	E × EGRQ	600	694	(115.7)	163
2	K × L	150	86	(57.3)	15
3	Olma × EGRQ	450	240	(53.3)	109
4	Kobra × A	450	218	(48.4)	94
5	D × Almari	600	241	(40.7)	94
6	W × EGRQ	450	150	(33.3)	13
7	A × Almari	600	168	(28.0)	77
8	F × Almari	600	117	(19.5)	3
9	P × Almari	150	26	(17.3)	8
10	M × Almari	150	25	(16.7)	6
11	R × EGRQ	150	24	(16.0)	18
12	O × Almari	150	21	(14.0)	0
13	Alba × EGRQ	450	55	(12.2)	48
14	Kraka × EGRQ	750	76	(10.1)	52
15	(B × Martin) × Almari	450	34	(7.5)	8
16	B × (C × Almari)	450	28	(6.2)	34
17	Jawa × EGRQ	300	10	(3.3)	22
18	SzD 3878 × Almari	300	9	(3.0)	19
19	H × Almari	450	13	(2.9)	46
20	Kobra × Z	450	5	(1.1)	9
21	G × Almari	450	2	(0.4)	35
22	Kobra × D	150	0	(0.0)	0
23	(N × Kraka) × Almari	600	0	(0.0)	0
24	(S × Juwel 15) × T	150	0	(0.0)	0
25	(B × Milan) × Almari	450	0	(0.0)	0

A total of 9900 anthers were plated to obtain 2242 green plants (73.0% in relation to anthers plated) and 885 (27.0%) albino plants (Fig. 2). The frequencies of somatic embryos and green plants were markedly influenced by the genotype. The anther culture of the O × Almari F_1 hybrid yielded only green

Fig. 1. Emergence of somatic embryos from *Triticum aestivum* after 4 weeks of culture

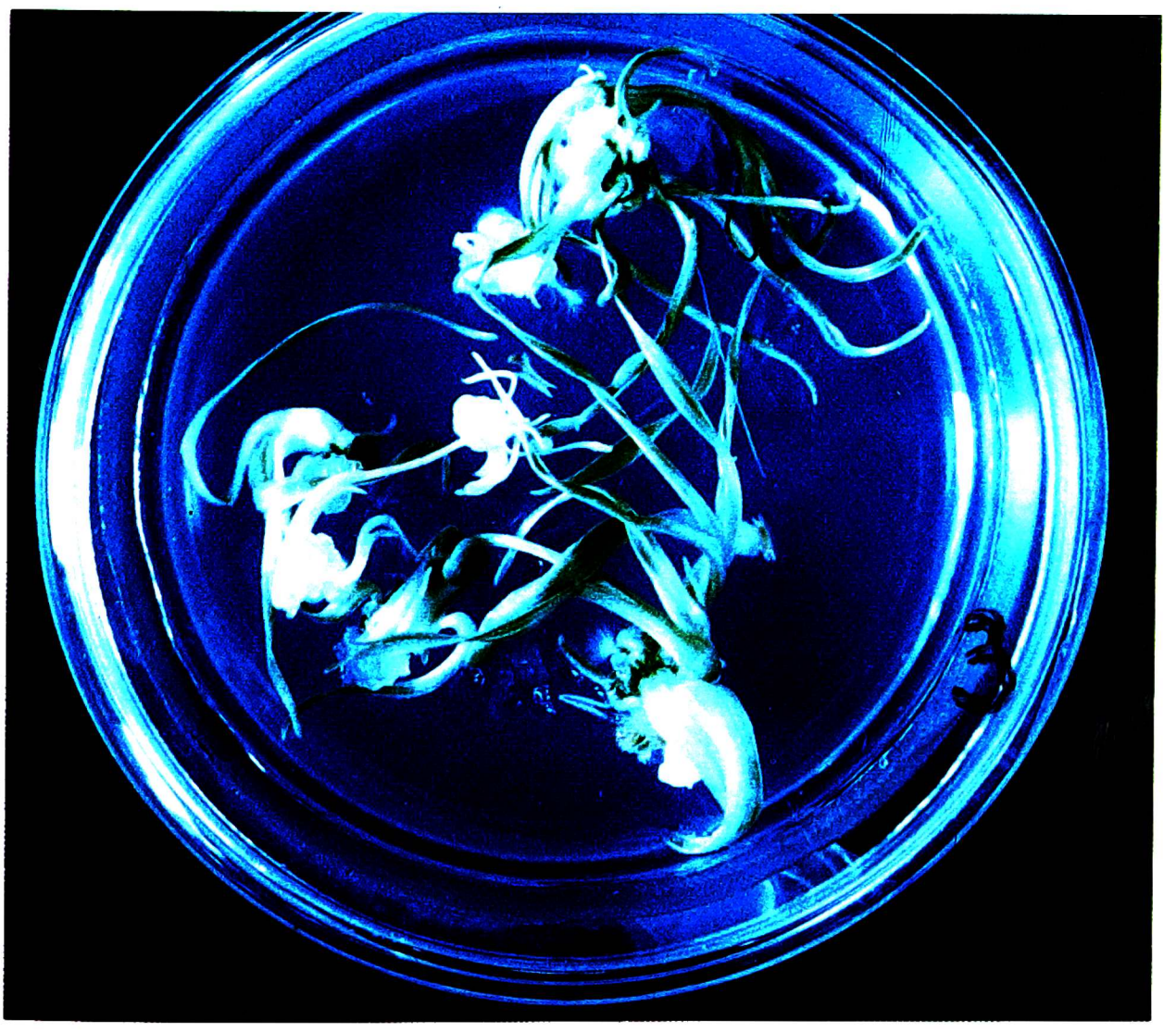

Fig. 2. Green and albino wheat plantlets development from somatic embryos after 6-8 weeks of culture

plants. The most responsive 14 F_1 hybrid combinations (Nos. 1-14, Table 2) produced more green than albino plants, 6 low responsive combinations (Nos. 15-21) yielded more albino than green plants, while 4 combinations (Nos. 22-25) produced no plants.

The highest percentage (115.7%) of green plants (694 green plants /600 anthers) was obtained from E × EGRQ genotype. 30-60% green plants were yielded by 5 genotypes (Olma × EGRQ, W × EGRQ, Kobra × A, K × L, D × Alma) 10-30% of green plants were produced by 9 genotypes (Kraka × EGRQ, Alba × ERGQ, R × EGRQ, A × Almari, G × Almari, F × Almari, M × Almari, O × Almari, P × Almari) and only 0.4-10% of green plants – by 6 genotypes (Kobra × Z, Jawa × EGRQ, B × C, (B × Martin) × Almari, H × Almari, SzD 3878 × Almari). EGRQ genotype was mostly involved in F_1 hybrid combinations which yielded the highest percent of green plants.

Table 3. Chromosomal constitution in a sample of 51 androgenic green plants prior to colchicine treatment

Chromosomal constitution	Plants	
	No.	(%)
Haploid (n=3x=21)	42	(82.4)
Diploid (2n=6x=42)	7	(13.7)
Aneuploid (2n=40, 41)	2	(3.9)

Somatic chromosome numbers were determined in 51 androgenic green plants (not treated with colchicine), representing most of the donor genotypes (Table 3). The majority of regenerants (82.4%) were haploids (n = 3x = 21), while 13.7% were spontaneous diploids (2n = 6x = 42) and 3.9% showed aneuploid chromosome numbers (2n = 40, 41).

Discussion

The presented results confirmed a great influence of genotype on wheat anther response. Green plant yield of the anther-donor 25 F_1 crosses ranged from 0 to 115.7%. EKIZ and KONZAK (1994) reported similar differences in the green plants yield (0-90%) for 44 varieties and 14 F_1 crosses of spring bread wheat. Embryo induction, plant regeneration and albino/green ratio in wheat anther culture were determined as heritable traits (AGACHE et al. 1989).

The three different processes might be under different genetic regulation and can be inherited independently (SZAKACS et al. 1988). The best-responding winter wheat cultivar "Florida" is characterized by the presence of a 1B/1R wheat-rye translocation chromosome (FOROUGHI-WEHR, ZELLER 1990). BULLOCK et al. (1982) pointed to the fact that the nuclear transferable character of in vitro androgenic capacity is important to plant breeders since anther cultures are used in breeding doubled haploids.

It was found for the genotypes studied that cold pretreatment of spikes stimulated the efficiency of androgenic response. The efficiency of androgenic somatic embryos induction was the highest when spikes were incubated at 4°C for 6-9 days. LAZAR et al. (1990) obtained the best results of androgenic response 7-14 days after cold pretreatment of spikes at 4°C. However, KARIMZADEH et al. (1995) found that cold treatment, generally, inhibited anther response and callus production in two wheat genotypes studied.

WANG and CHEN (1980), JONES and PETOLINO (1987) emphasized the importand of temperature during growth of donor plants as well as their physiological condition.

The frequency of androgenic embryos and regeneration of green plants depends also on the temperature of anther cultures. According to OUYANG et al. (1983) suitable temperatures for anther culture in wheat are between 26°C and 30°C.

A specific feature of wheat anther cultures is a high percentage of albinotic plants. However, the problem of albinos occurrence in monocotyledons has not been decisively solved yet. HUANG (1987) suggests that the ratio of green to albinotic plants depends on the culture temperature. The number of albino plants is known to be influenced by the genotype (OUYANG et al. 1983, ANDERSEN et al. 1987, HASSAWI et al. 1990). NAVARRO-ALVAREZ et al.(1994) suggested that sugars used in initiation and regeneration media with wheat starch affect the frequency of albinos. ZIEGLER et al. (1990) showed that the ratio of green to albino plants can be improved by decreasing light intensity during the regeneration phase.

Chromosome counts of root tip cells showed that the majority of green plants were haploids (82.4%), however, only 13.7% were spontaneous dihaploids and 3.9% aneuploids plants. BULLOCK et al. (1982) examined 24 plants and found 21 haploids (87.5%) and only three dihaploids (12.5%). ZIEGLER et al. (1990) reported 78.3% of haploids and only one diploid and four mixoploid plants out of 23 green regenerants. However, METZ et al. (1988) and MASOJĆ et al. (1993) found higher proportions of spontaneous diploids, viz. 29% and 41%, respectively, and about 45-50% of haploids.

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