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THE EFFECT OF PHYSICAL MEDIUM STATE ON ANTHER CULTURE RESPONSE IN POLISH CULTIVATED OAT (*AVENA SATIVA* L.)

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The effect of solid, liquid and double-layer W14 induction media on androgenic response and plant regeneration from 15 F_3 generations of hexaploid oat hybrids was investigated. Embryo-like structures (ELS) were obtained from eight genotypes (average 1.4/100 anthers), of which six showed response on media in all physical states. The frequency of embryo induction (6.6/100 anthers) was highest in genotype CHD1780/05 on solid medium. Plants were regenerated from only two genotypes: CHD1780/05 (2.2 plants/100 anthers) and CHD1989/05 (1.3 plants/100 anthers). A total 35 plants (22 of CHD1780/05, 13 of CHD1989/05) were regenerated only from ELS obtained on solid medium.

Key words: *Avena sativa*, anther culture, medium state, androgenic plants, embryo-like structures.

INTRODUCTION

The application of anther culture in plant breeding depends on the production of a large number of haploid plants and on a high frequency of their chromosome doubling. Oat is one of the most recalcitrant cereal species for anther culture, and practical application in breeding is still limited by the low frequency of ELS induction and plant regeneration. Haploid plants of oat can be obtained through anther culture or through crossing with maize. There are several publications on haploid production through the oat × maize (*Zea mays* L.) system (Rines and Dahleen, 1990; Matzk, 1996; Riera-Lizarazu et al., 1996; Sidhu et al., 2006). Oat haploids have been produced mainly by anther culture. Various factors determining the anther culture response and production of androgenic plants have been investigated, including genotype, pretreatment of spikes, media composition and culture environment (Rines, 1983; Kiviharju et al., 1997; Kiviharju and Puolimatka, 1998; Kiviharju and Pehu, 1998; Kiviharju and Tauriainen, 1999; Kiviharju et al., 2000; 2005).

The present study compares the effects of using solid, liquid and double-layer W14 induction media on androgenic response and plant regeneration from 15 Polish oat genotypes.

MATERIALS AND METHODS

Fifteen F3 generations of hexaploid oat hybrids were used: CHD: 1705/05, 1717/05, 1725/05, 1780/05, 2038/05, 1889/05, 1893/05, 1903/05, 1944/05, 1954/05, 1956/05, 1967/05, 1985/05, 1989/05 and 1997/05 (DANKO Plant Breeders, Choryń, Poland). Donor plants were grown in a greenhouse. Tillers were harvested when most microspores were at the uninucleate stage and cold-treated at 4°C for 6–9 days in N_6 mineral salt medium (Chu et al., 1975) with 2.0 mg/l 2,4-D. After cold-pretreatment, spikes were surface-sterilized with 5% calcium hypochlorite for 8 min, then washed several times with sterile distilled water.

Anthers were isolated aseptically and transferred to Petri dishes (5 cm diam) with liquid, solid or double-layer induction medium containing W14 salts and vitamins (Ouyang et al., 1989) with 5.0 mg/l 2,4-D + 0.5 mg/l BAP + 20.0 mg/l Ethephon + 50.0 mg/l L-cysteine + 500.0 mg/l myo-inositol (modification by Kiviharju et al., 2005). The solid medium, containing 6 g/l agarose, was sterilized by autoclaving at 120°C for 20 min. The liquid medium, containing 10% Ficoll 400, was sterilized by filtration. The double-layer medium employed the above-mentioned solid and liquid media. About 170 anthers were collected per Petri dish, and 1,000 anthers of each genotype were isolated on each

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TABLE 1. Two-way ANOVA for effects of induction medium and genotype on embryo-like structure production in oat

Source of variation	d.f.	Mean square
Genotype (G)	14	3.225*
Medium (M)	2	1.508
MxG interaction	28	1.882*
Error	90	0.586

*p<0.01

medium. The Petri dishes were sealed with parafilm and then placed in a dark incubation chamber at 28°C. ELS were transferred to 190-2 solid regeneration medium (Zhuang and Xu, 1983) and then incubated under fluorescent light at 22°C under a 16 h photoperiod. Green plant yield was determined as the number of green plants obtained from 100 plated anthers. Plants were potted and grown in a greenhouse. ELS development was determined in anther squashes with acetocarmine.

STATISTICS

Two-way ANOVA was performed to study the effects of genotype, induction medium, and medium x genotype interaction. Only the data for embryo-like structures were included in statistical calculations because of the high number of zero values for number of green and albino plants regenerated. Before ANOVA the data were arcsine transformed to normalize the distribution. Duncan's test was used to compare the androgenic response of the studied genotypes.

RESULTS

In this study, multicellular pollen grains were observed after three weeks of culture (Fig. 1). ELS appeared after six weeks of culture on liquid medium (Fig. 2) and between the seventh and eighth weeks on solid and double-layer medium (Fig. 3).

Analysis of variance showed significant differences between the genotypes in the formation of embryo-like structures. The effect of medium state on this trait was not significant, but genotype × medium interaction was important (Tab. 1).

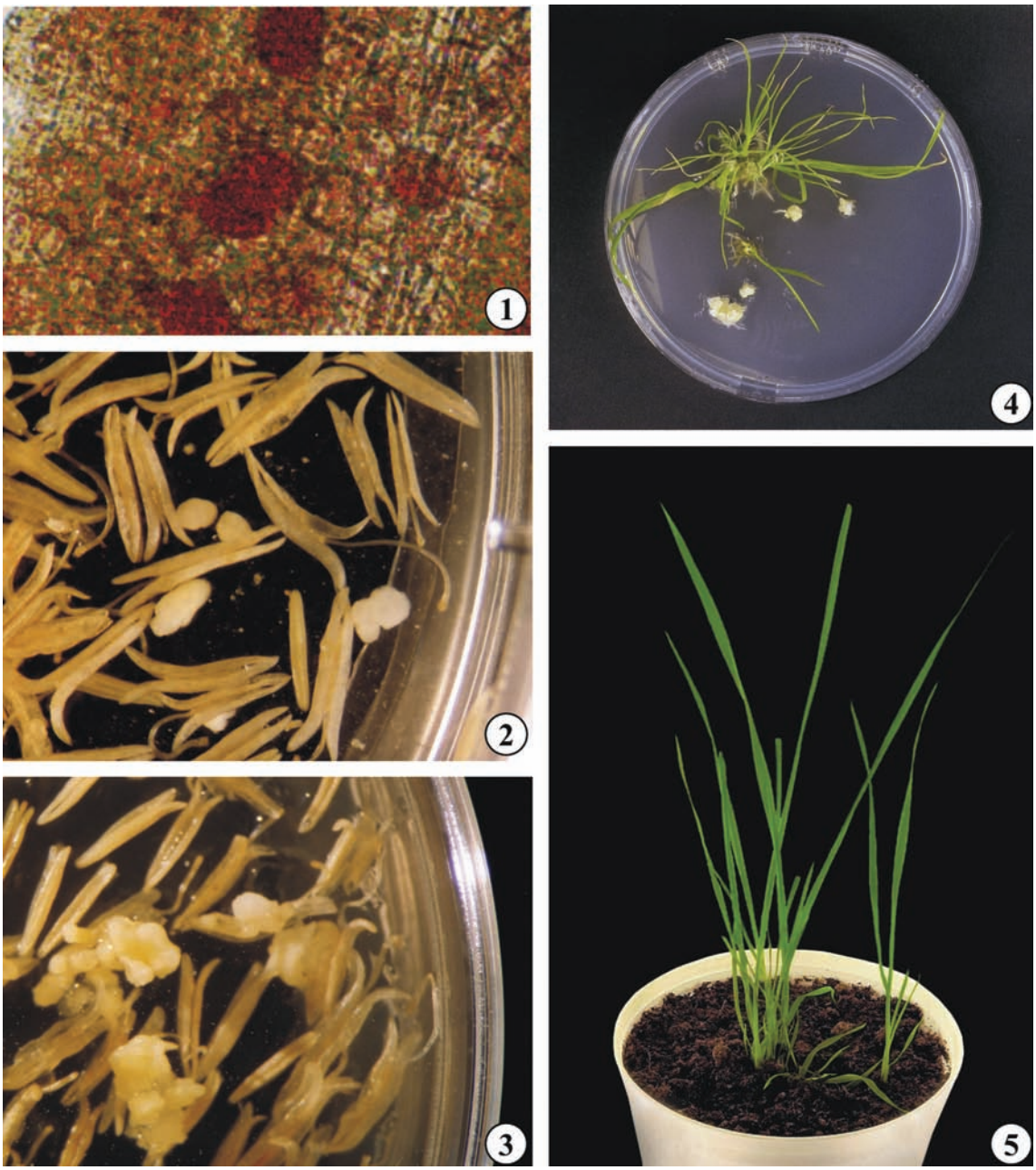
The frequencies of ELS formed on solid, liquid and double-layer media are compared in Tables 2 and 3. Of 45,000 anthers plated during this experiment, 637 ELS (1.4%) were produced on W14 medium in all three physical states. The frequencies of ELS and green plants were markedly influenced by genotype. ELS were obtained from eight genotypes (average 1.4/100 anthers), of which six responded on medium in all physical states.

Eight of the 15 genotypes tested were capable of ELS induction and development, and six genotypes responded on all the media used. Induction efficiency was higher on solid medium, on which 307 ELS were produced (1.1–6.6% depending on genotype), as compared to 173 ELS (0.5–4.8%) on liquid and 157 ELS (1.0–4.9%) on double-layer media. Two genotypes gave the best embryo induction rates on all three forms of medium: CHD1780/05 (3.2–6.6% ELS depending on medium) and CHD1967/05 (4.8–5.7% ELS depending on medium). Seven genotypes did not respond on any medium. Only ELS obtained on solid medium produced green rooted plants; a total 35 were regenerated from only two genotypes: 13 plants of CHD1989/05 (1.3/100 anthers or 25.0/100 ELS) and 22 plants of CHD1780/05 (2.2/100 anthers or 33.3/100 ELS) (Figs. 4, 5). No green plants were regenerated from the liquid and double-layer media treatments. It should be stressed that a relatively high number of ELS did not always result in a high number of green plants. For example, for ELS CHD1967/05, 5.7 ELS (solid media), 4.8 ELS (liquid) and 4.9 ELS (double-layer) per 100 anthers were formed, from which no plant was regenerated.

DISCUSSION

Not many papers on oat androgenesis have been published. Rines (1983) reported regeneration of three anther-derived plants, while Sun et al. (1991) recovered 12 green plants of naked oat. Kiviharju et al. (1997) regenerated anther-derived plantlets from wild oat, also with low frequency. Induction of embryo-like structures has been improved by Kiviharju and Puolimatka (1998), Kiviharju and Pehu (1998), Kiviharju and Tauriainen (1999) and Kiviharju et al. (2000). Only Kiviharju et al. (2005) described high frequency of ELS induction and plant regeneration in cultivated oat.

Our study assessed the effects of solid, liquid and double-layer W14 induction medium as modified by Kiviharju et al. (2005) on androgenic response and plant regeneration in 15 Polish cultivated oat hybrids. Although the influence of medium state on anther culture response was not statistically significant, the results showed that anthers incubated on solid medium yielded more ELS than those incubated in liquid and double-layer culture. The response of anthers depended mainly on the genotype used. Plantlets were regenerated only from ELS obtained on solid medium, and not from ELS cultured on medium in liquid or double-layer form. In contrast, Kiviharju et al. (2005) found that the double-layer form of modified W14 medium gave a very high frequency of androgenic embryo induction and plant regeneration, and that the results also depended on the genotype. For wheat, Puolimatka and Pauk (2000) found that using double-



Figs. 1–5. Development of ELS and plant regeneration of oat from genotype CHD1780/05. **Fig. 1.** Multicellular pollen grains in anther squashes after three weeks of culture. × 200. **Fig. 2**. ELS in liquid induction medium after six weeks. × 7. **Fig. 3**. ELS on solid induction medium after eight weeks. x 7. **Fig. 4**. Plantlets developed from androgenic ELS on regeneration medium 190-2 after four weeks. **Fig. 5**. Plants transferred to soil.

layer technique or adding Ficoll did not improve the androgenic response versus that with original liquid W14 medium. For triticale, Immonen and Robinson (2000) compared the efficiency of haploid production in liquid and solid media using W14 macro- and microelements with MS vitamins and iron, and demonstrated that liquid medium with Ficoll promoted a three- to fourfold increase in induction in one of the three triticale cultivars tested. Ponitka and Ślusarkiewicz-Jarzina (2007) investigated the effect of solid and liquid C17 induction media on anther response in triticale. In all tested genotypes the efficiency of ELS and green plant production was higher on liquid medium.

The results of our studies so far on production of oat haploids and DH lines do not permit a definite

TABLE 2. Effect of genotype on embryo-like structure induction and plant regeneration on solid W14 medium in oat

Genotype	ELS (n)	ELS/ 100 anthers	Green plants (n)	Green plants/ 100 anthers	Green plants/ ELS (%)	Albino plants (n)	Albino plants /100 anthers
CHD1705/05	13	1.3 ab	0	0	0	0	0
CHD1717/05	21	2.1 bc	0	0	0	2	0.2
CHD1725/05	0	0 a	0	0	0	0	0
CHD1780/05	66	6.6 e	22	2.2	33.3	3	0.3
CHD2038/05	0	0 a	0	0	0	0	0
CHD1889/05	39	3.9 d	0	0	0	0	0
CHD1893/05	29	2.9 de	0	0	0	3	0.3
CHD1903/05	0	0 a	0	0	0	0	0
CHD1944/05	0	0 a	0	0	0	0	0
CHD1954/05	30	3.0 c	0	0	0	1	0.1
CHD1956/05	0	0 a	0	0	0	0	0
CHD1967/05	57	5.7 e	0	0	0	0	0
CHD1985/05	0	0 a	0	0	0	0	0
CHD1989/05	52	5.2 e	13	1.3	25.0	2	0.2
CHD1997/05	0	0 a	0	0	0	0	0

Different letters in one column represent significant differences at $p < 0.05$

TABLE 3. Effect of genotype on anther culture response on liquid and double-layer W14 medium in oat

Genotype	Liquid medium				Double-layer medium			
	ELS (n)	ELS/100 anthers	Albino plants (n)	Albino plants/ 100 anthers	ELS (n)	ELS/100 anthers	Albino plants (n)	Albino plants/ 100 anthers
CHD1705/05	5	0.5 ab	0	0	10	1.0 ab	1	0.1
CHD1717/05	7	0.7 ab	1	0.1	0	0 a	0	0
CHD1725/05	0	0 a	0	0	0	0 a	0	0
CHD1780/05	41	4.1 e	0	0	32	3.2 c	0	0
CHD2038/05	0	0 a	0	0	0	0 a	0	0
CHD1889/05	16	1.6 b	2	0.2	15	1.5 b	1	0.1
CHD1893/05	0	0 a	0	0	12	1.2 ab	0	0
CHD1903/05	0	0 a	0	0	0	0 a	0	0
CHD1944/05	0	0 a	0	0	0	0 a	0	0
CHD1954/05	21	2.1 b	1	0.1	17	1.7 b	2	0.2
CHD1956/05	0	0 a	0	0	0	0 a	0	0
CHD1967/05	48	4.8 c	1	0.1	49	4.9 d	3	0.3
CHD1985/05	0	0 a	0	0	0	0 a	0	0
CHD1989/05	35	3.5 c	0	0	22	2.2 bc	0	0
CHD1997/05	0	0 a	0	0	0	0 a	0	0

Different letters in one column represent significant differences at $p < 0.05$

judgement on whether the use of double-layer or solid medium is more advantageous for anther culture. Our study used W14 medium as modified by Kiviharju et al. (2005) but did not yield similar results for Polish oat cultivars. Further experiments are needed: successive modification of anther culture media and conditions may increase the efficiency of ELS production and plant regeneration in oat.

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