PRODUCTION OF HAPLOIDS AND DOUBLED HAPLOIDS OF THE AMPHIPLOIDS *AEGILOPS VARIABILIS* × *SECALE CEREALE*

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Summary: Anther culture was used to produce haploid plants from *Aegilops variabilis ssp. euvariabilis* × *Secale cereale* (inbred line) amphiploids (2n=42) and their parental forms. The anthers were cultured on modified induction media - C_{17} and PII. Androgenetic embryos were obtained only from amphiploid plants and the frequency depended on genotypes. On C_{17} medium 20.0 - 65.2 and on PII medium 1.4 - 15.7 androgenetic embryos /100 anthers were produced. The embryos were transferred to a regeneration medium 190-2 and 0.1 - 13.4 of green plants /100 androgenetic embryos were obtained. The haploid (n=21) chromosome number was found in all plants. Doubled haploids were produced by haploid colchicine treatment.

Key words: anther culture, amphiploids, *Aegilops variabilis,* doubled haploids, haploids, androgenetic embryos, *Secale cereale*

Introduction

Haploid and doubled haploid plants have many uses in genetic studies and in plant breeding. Within the genus *Aegilops* spontaneous haploids have been reported for diploid species *Aegilops squarrosa* L. (Dosba et al., 1979), *Aegilops caudata* L. (Chapman and Riley, 1964), and *Aegilops longissima* Schwein. et Musch. (Riley and Chapman, 1957) as well as for the tetraploid species *Aegilops ovata* L. (Matsumura, 1940). Haploid plants in *Aegilops crassa* Boiss., *Aegilops triuncialis* L. (Chapman and Miller, 1977) and *Triticum ventricosum* Ces. (Fedak, 1983) were obtained by pollinated with pollen from plants of tetraploid *Hordeum bulbosum.* In rye, spontaneous haploids were reported by Levan (1942) and Heneen (1962). Haploids of rye were also obtained by anther culture (Deimling and Geiger, 1996; Immonen and Anttila, 1998; Rakoczy-Trojanowska et al., 1997; Ponitka and Ślusarkiewicz-Jarzina, unpublished) and microspores (Guo and Pulli, 2000). Attempts to produce rye haploids through chromosome elimination have failed so far. Crosses between *Secale cereale* L. and *Zea mays* L., *Hordeum bulbosum* L., *Dactylis glomerata* L. and *Festuca glauca* L. generated mostly globular embryos (Zenkteler and Nitzsche, 1984; Bolesta et al., 1997; Ponitka and Ślusarkiewicz-Jarzina, unpublished).

The aim of this work was to study the androgenic response and production of haploids from the *Aegilops variabilis ssp. euvariabilis* × *Secale cereale* (inbred line) amphiploids (2n=42) and parental forms, as well as production doubled haploids.

Material and methods

Anthers were obtained from the *Aegilops variabilis ssp. euvariabilis* × *Secale cereale* (inbred line No 2480k) amphiploids (2n=42) and parental forms. The amphiploids 408C, 408D, 408E were produced by *in vitro* F_1 hybrid propagation of different explants, the amphiploid 408B was obtained through colchicine treatment of the hybrids (Wojciechowska and Pudelska, 1999). Generations 408B - C_4, 408C, 408E - R_2 and 408D - R_1 were used in this experiment.

Plants were grown in the greenhouse. Spikes were cut at the uninuclear stage of microspore development and kept at 4°C for 6-9 days in mineral salt medium N_6 (Chu et al., 1975). After sterilization in 5% calcium hypochlorite, anthers at the mid-uninucleate stage were plated on induction media:

- C_{17} (Wang and Chen, 1983) + 90 gL^{-1} maltose (instead of sucrose)
- PII (Chuang et al., 1978) + 0.5 mgL^{-1} 2,4-D + 90 gL^{-1} maltose (instead of sucrose).

The anthers were incubated in darkness at 30°C. After 4 weeks, embryos developed from the microspores were transferred to a regeneration medium 190-2 (Zhuang and Xu, 1983) and illuminated for 16 h/day at 22°C. Green plantlets were transferred into pots and their chromosome numbers were determined in root-tip squashes. Roots were pretreated with ice-cold water for 24 h, fixed in ethanol-acetic acid (3:1) and stained with Feulgen. After vernalization (8 weeks at 4°C), green haploids at the five-leaf stage were placed in a solution comprised of 0.1% colchicine + 4 mg L^{-1} DMSO + Tween 20 + 25 mg L^{-1} GA_3 and kept for 12 h in continuous light at 22°C. Excess colchicine was removed by washing their roots for 2 h with tap water and the plants were finally transplanted to pots. The stainability of pollen grains was determined using solution of acid fuchsin in lactophenol (Sass, 1964).

Results and discussion

In this study, the first nuclear and cell divisions of microspores were observed between the third and fifth day of anther culture. Multicellular grains were observed after two weeks (Figure 1) and androgenetic embryos appeared after four weeks. Androgenetic embryos developed only in amphiploids and *Aegilops variabilis* cultures. The frequencies of androgenetic embryos and of regenerated plants are shown in Table 1. Induction efficiency was higher in medium C_{17} which produced 20.0 – 65.2 embryos/100 anthers, compared to medium PII with 1.4 – 15.7 embryos /100 anthers. Higher efficiency of medium C_{17} in androgenetic embryo production was previously reported for triticale (Ponitka et al., 1999).

Green plants were obtained from two amphiploids, 408C and 408B, with a frequency of 0.1 – 13.4 plants /100 androgenetic embryos. Chromosome counts in root-tip cells showed that all green plants were haploid (n=21). Albino plants developed in all amphiploid cultures and the frequency ranged from 1.9 to 37.5 /100

androgenetic embryos (Figure 2). No green or albino plants were obtained from the parental species, *Aegilops variabilis* and *Secale cereale*.

Low efficiency of anther culture for *Aegilops* haploid production has been reported in other experiments (Dosba et al., 1979; Chapman and Riley, 1964; Riley and Chapman, 1957; Matsumura, 1940; Chapman and Miller, 1977; Fedak, 1983). In rye, the frequency of anther-derived haploid plants strongly depended on genotype, with the best results, 20.0 and 11.0 green plants /100 anthers, obtained by Immonen and Anttila (1998), respectively. In our study, most of the green plants, 13.4 /100 anthers, were obtained from the amphiploid 408C (Table 1). In hybrids of wheat (Tuvesson et al., 1989) and triticale (Charmet and Bernard, 1984; Ślusarkiewicz-Jarzina et al., 1996), the induction rate, plant regeneration and green plant yield were also higher than in parental forms.

Table 1. Androgenetic embryos formation and plant regeneration frequencies in anther culture of *Aegilops variabilis* × *Secale cereale* amphiploids and parental forms

Genotype	Induction medium	Anthers plated No.	Androgenetic embryos No. (%)	Green plants No. (%)	Albino plants No. (%)
408B	C_{17}	1128	736 (65.2)	1 (0.1)	16 (2.2)
	PII	630	53 (8.4)	0	0
408C	C_{17}	516	291 (56.0)	39 (13.4)	7 (2.4)
	PII	504	79 (15.7)	0	0
408D	C_{17}	583	195 (33.4)	0	0
	PII	556	8 (1.4)	0	3 (37.5)
408E	C_{17}	528	106 (20.0)	0	2 (1.9)
	PII	429	0	0	0
Aegilops variabilis	C_{17}	811	1 (0.1)	0	0
	PII	657	0	0	0
Secale cereale	C_{17}	530	0	0	0
	PII	620	0	0	0

After colchicine treatment, 33 plants of the 408C amphiploid survived. Out of 33 plants, 18 were sterile and did not set seed, while the rest of the plants were chimeric and set some seeds. The chimeric plants exhibited good tillering capacity:

from 47 to 110 culms, as compared to 37 to 56 culms from 408C plants. Dehisced anthers were observed only in some spikes. The stain ability of the pollen grains was 65.0% while in initial plants – 52.6%. Seed set of doubled tillers varied from 1 to 18 per spike (Table 2) and from 1 to 154 per plant. The initial plants seed set ranged from 1 to 10 seeds per spike and 55 to 168 seeds per plant. A total of 15 (38.5%) doubled haploid lines were obtained from the amphiploid 408C. Production of these lines widened the genetic material for study the Ae. variabilis x S. cereale amphiploids, especially their fertility in successive generations and cross ability with rye.

Table 2. Frequency of seed set in doubled haploid plants of the 408C amphiploid *Aegilops variabilis x Secale cereale*.

Plant number	Tillers No.	Spikes with seeds		
		Total No. (%)	From 1 to 7 No.	From 8 to 18 No.
532	55	7 (12.7)	3	4
533	60	5 (8.3)	3	2
534	110	1 (0.9)	1	0
535	100	4 (4.0)	1	3
537	53	6 (11.3)	2	4
538	61	19 (31.1)	9	10
539	77	2 (2.6)	2	0
541	92	1 (1.1)	1	0
543	84	1 (1.2)	1	0
544	79	1 (1.3)	1	0
545	86	1 (1.2)	1	0
546	71	2 (2,8)	2	0
548	48	1 (2.1)	1	0
563	47	5 (10.6)	0	5
564	50	1 (2.0)	1	0

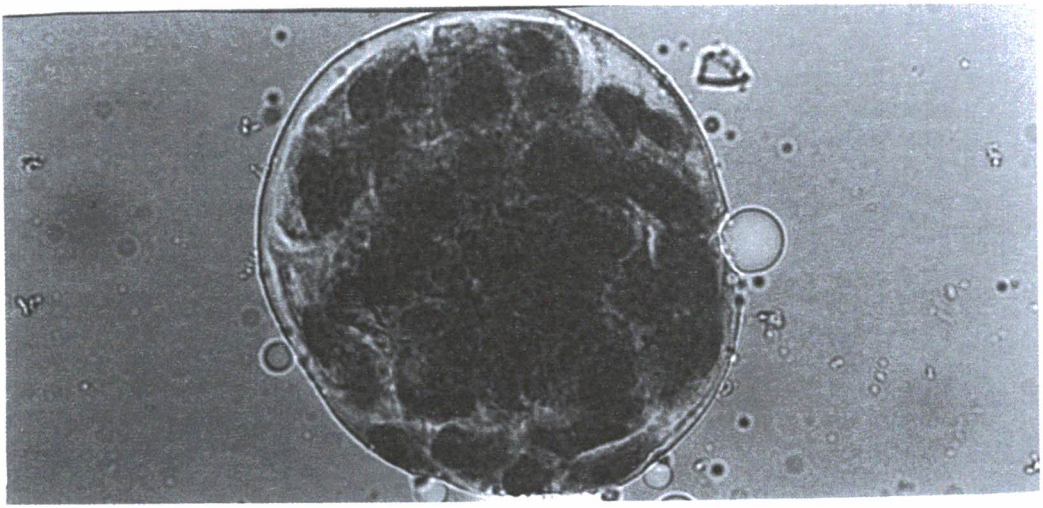

Figure 1. Embryoid of the amphiploid 408C after two weeks anther culture on the C_{17} induction medium

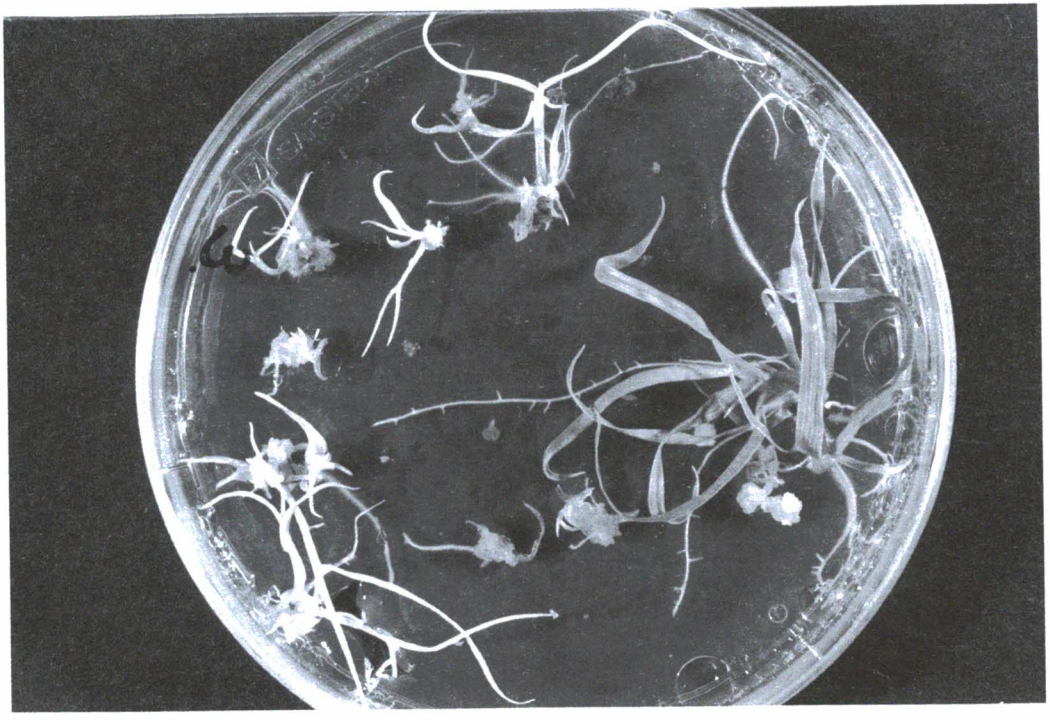

Figure 2. Green and albino amphiploid 408C plantlets from androgenetic embryos after two weeks on the 190-2 regeneration medium

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