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Effect of genotype and media composition on embryoid induction and plant regeneration from anther culture in triticale

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Abstract. Anthers of twenty triticale genotypes were cultured on three different media: 1 – PII (CHUANG et al. 1978) with increased 2,4-D to 2 mg L^{-1} and agarose 6 g L^{-1}; 2 – Macro-, micronutrients and vitamines like in MN6 (CHU, HILL 1988) + 2 mg L^{-1} 2.4-D + 0.5 mg L^{-1} KIN + 5 mg L^{-1} FeEDTA + 90 g L^{-1} sucrose; 3 – Macro-, micronutrients and vitamines like in MN6 (CHU, HILL, 1988) + 2 mg L^{-1} 2.4-D + 0.5 mg L^{-1} KIN + 5 mg L^{-1} FeEDTA + 120 g L^{-1} sucrose. Embryoid induction and plant regeneration were influenced by donor plant genotype and induction medium. Medium 1 was the best for embryoid induction, while for green plant regeneration the best were media 1 and 2. Out of 300 anthers from each genotype plated on each of the three media, 64-1250, 12-486 and 6-212 somatic embryos and 8-86, 3-136 and 1-26 green plants were recorded, on media 1, 2 and 3, respectively.

Key words: anther culture, haploid plants, somatic embryos, triticale.

Introduction

Since the first report on haploid plant production from cultured anthers of triticale by WANG et al. (1973), the practical application of androgenetic haploids is still limited due to the small number of haploid individuals obtained. Several studies have identified genetic factors (CHARMET, BERNARD 1984), cultivation conditions of donor plants (BERNARD 1977), developmental stage of microspores (HASSAWI, LIANG 1990), pretreatment of spikes (SOZINOV et al. 1981, SHARMA et al. 1982), media composition (SHARMA et al. 1982, WANG, HU 1984, KLEIJER 1991, KARSAI et al. 1994) and culture environments

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(BERNARD 1980, HASSAWI, LIANG 1990) as the factors that affect callus initiation and plant regeneration.

The aim of the present work was to study the androgenic response and production of haploid plants from different triticale genotypes on three induction media.

Material and metods

Anther-donor plants were F_1 hybrids of hexaploid triticale obtained from the Plant Breeding Station of Szelejewo, Poland. The plants were grown in the field. The spikes were cut at the uninuclear stage of microspore development, which was determined cytologically. Before being plated on the medium, the spikes were kept at 4°C for 6-9 days in the mineral salt medium N6 (CHU et al. 1975). After sterilization in 5% calcium hypochlorite, anthers at the mid-uninucleate stage were plated on three induction media:

1. PII (CHUANG et al. 1978) with increased 2,4-D to 2 mg L^{-1} and agarose 6 g L^{-1};
2. Macro-, micronutrients and vitamines like in MN6 (CHU, HILL 1988) + 2 mg L^{-1} 2.4-D + 0.5 mg L^{-1} KIN + 5 mg L^{-1} FeEDTA + 90 g L^{-1} sucrose;
3. Macro-, micronutrients and vitamines like in MN6 (CHU, HILL 1988) + 2 mg l^{-1} 2.4-D + 0.5 mg L^{-1} KIN + 5 mg L^{-1} FeEDTA + 120 g L^{-1} sucrose.

On each of the three induction media 300 anthers were plated, a total of 900 anthers from each of the twenty genotypes. The anthers were incubated in darkness at 30°C. After 3-4 weeks, the somatic embryos developed from microspores were transferred to a regeneration medium 190-2 (ZHUANG, JIA 1983) and illuminated for 16 h/day. Green plantlets were then transferred into tubes containing the same regeneration medium and subsequently transferred into pots. After vernalization (8 weeks at 4°C) green haploids at five-leaf stage of development were placed in a solution of 0.1% colchicine + 4 mL 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 chromosome number of the plants developed in vitro was determined in root-tip squashes with acetocarmine.

Results

Embryonic structures (Fig. 1) in the burst anthers were observed after 3 or 4 weeks of culture. Table 1 shows the frequencies of embryonic structures formation for twenty genotypes on three induction media.

Fig. 1. Embryos emerging from triticale anthers after 4 weeks of culture

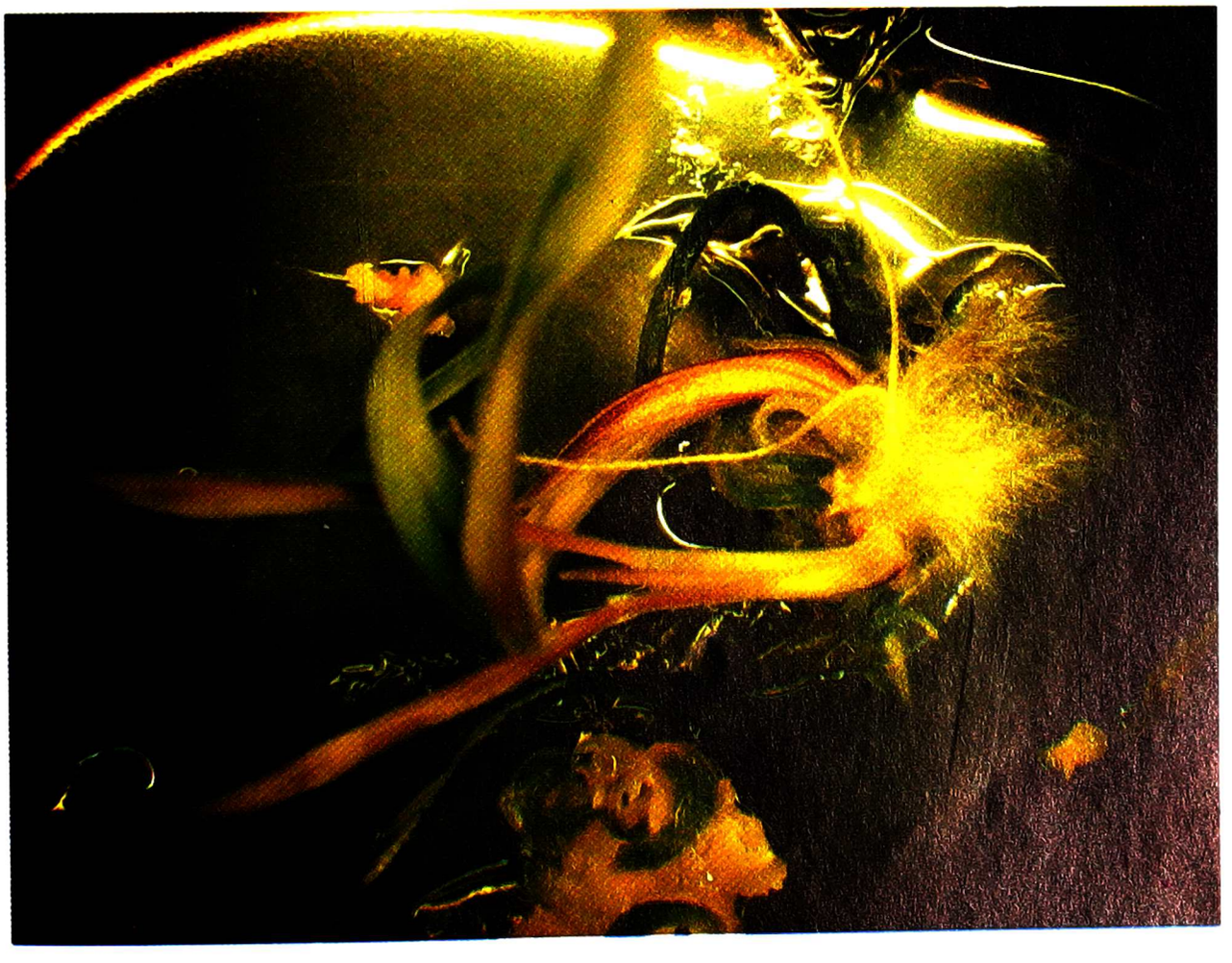

Fig. 2. Green triticale plantlets developed from somatic embryos after 6-8 weeks of culture

Table 1. Embryoid formation and plant regeneration from triticale anthers on three induction media

Geno-type	Medium 1			Medium 2			Medium 3		
	somatic embryos	plants		somatic embryos	plants		somatic embryos	plants	
	no.	green no. (%)	albino no.	no.	green no. (%)	albino no.	no.	green no. (%)	albino no.
57	362	82 (27.3)	181	246	29 (9.7)	41	165	26 (8.7)	51
188	241	22 (7.3)	50	70	3 (1.0)	8	13	–	–
192	–	–	–	17	–	4	17	1 (0.8)	2
205	170	54 (18.0)	11	98	6 (2.0)	–	54	–	7
276	851	34 (11.3)	332	486	136 (45.3)	70	95	–	16
290	172	42 (14.0)	–	148	50 (16.7)	32	16	2 (0.7)	6
309	64	–	–	21	8 (2.7)	–	–	–	–
310	142	8 (2.7)	48	276	60 (20.0)	38	33	6 (2.0)	–
340	1250	60 (20.0)	456	314	16 (5.3)	88	87	6 (2.0)	12
352	371	29 (9.6)	86	103	3 (1.0)	29	158	–	53
376	422	54 (18.0)	135	150	16 (5.3)	44	210	4 (1.3)	56
397	370	52 (17.3)	22	62	–	–	6	–	–
406	442	86 (28.7)	82	261	52 (17.3)	26	51	–	–
436	261	40 (13.3)	28	215	64 (21.3)	6	–	–	–
444	376	60 (20.0)	48	32	–	–	–	–	–
452	152	41 (13.6)	39	157	57 (19.0)	19	111	3 (1.0)	19
464	137	10 (3.3)	24	342	72 (24.0)	44	84	2 (0.7)	8
Szd 1740	481	38 (12.6)	84	191	10 (3.3)	34	212	6 (1.9)	64
Szd 1745	490	36 (12.0)	74	78	11 (3.5)	9	95	15 (5.0)	–
Mah 16118-6	162	–	4	12	–	–	68	6 (2.0)	–
Total	6916	748 (12.5)	1704	3278	593 (9.9)	492	1475	77 (1.3)	294

% of green plants calculated in relation to the number of anthers plated

Medium 1 was more efficient than media 2 and 3 for somatic embryo induction. Large differences in somatic embryo initiation frequency were found among the twenty genotypes. Seventeen genotypes showed the highest initiation frequency on medium 1, while the other three genotypes showed the highest frequency on medium 2. Out of the total 18,000 anthers cultured, 1,418 green and 2,490 albino plants were regenerated. Out of the 6,000 anthers (300 of each genotypes) plated on each of the three media, 748 (12.5%), 593 (9.9%) and 77 (1.3%) green plants were obtained on media 1, 2 and 3, respectively. The number of green plants depended also on donor genotypes. The percent of green plants from 20 genotypes was 2.7-28.7 on medium 1, 1.0-45.3 on medium 2 and 0.7-8.7 on medium 3. The highest percent of green plants (Fig. 2) was produced by genotype No. 276 on medium 2, although on this medium the average number of green plants was lower than on medium 1. Media 1 and 2 contained 9% sucrose and were the most suitable for androgenic response despite considerable differences in the other components. Medium 3, which contained 12% sucrose but was otherwise the same as medium 2, was much less suitable for androgenic response. A high rate of albino plantlets was observed in the regenerated plants (69.5%, 45.3%, 79.2% albinos on media 1, 2 and 3, respectively).

Table 2. Chromosomal constitution in a random sample of 58 pollen plants of triticale prior to colchicine treatment

Chromosomal constitution	No. (%) of plants
Haploid (n=3x=21)	53 (91.38)
Diploid (2n=6x=42)	2 (3.45)
Aneuploid (2n=22, 40)	2 (3.45), 1(1.72)

Fiftythree of 58 regenerated green plantlets (91.38%) represented polyhaploids. Also diploids and aneuploids occurred spontaneously among the 58 regenerated plantlets (Table 2).

Discussion

Results showed the importance of genotype, medium composition and the interaction beetwen them for the success of anther culture of triticale.

The embryoid induction medium is one of the key factors determining anther culture response in triticale. MARTINEZ GARCIA et al. (1992) reported on the different response of genotypes on two induction media with respect to embryo/callus production, plant regeneration and green plant production. In our study the efficiency of somatic embryo induction was the highest on medium 1 (potato II). WANG and HU (1984) recorded a better androgenic response on potato II medium than on B5 medium.The number of developed embryos and plants was significantly affected by induction media 1 and 2.

In our study, differences among the triticale genotypes of the applied media were clearly recognizable.

Genotypic response can be modified by different induction media. We found that the concentration of sucrose in the medium is an important factor inducing androgenesis and influencing the frequency of green plant regeneration. Sucrose is used as a source of carbohydrates for the developing structures and as a source of conditioning osmotic potential. KARSAI et al. (1994) obtained high embryoid induction of triticale on MN6 medium using maltose instead of sucrose.

In triticale the percentage of green plantlets is under genetic control. For this reason a better understanding of genotypic factors influencing the androgenic process may improve anther culture efficiency. CHARMET and BERNARD (1984) and ŚLUSARKIEWICZ-JARZINA et al. (1996) found that additive and dominance effects contributed to anther culture response. In triticale albinism remains a serious problem. Most of the plantlets produced in this study were albinotic. High rates of albino plantlets were also reported by BERNARD (1977), SOZINOV et al. (1981) and SCHUMANN (1990). However, most of the regenerated green plants were haploids, as in the work of SCHUMANN (1990) and KLEIJER (1991).

In conclusion, our results clearly demonstrate that, by modifying medium composition, it was possible to notably increase the success rate of in vitro androgenesis in triticale.

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