(From the Institute of Microbiology and Genetics of the University of Vienna)

Anther culture responsiveness of austrian winter wheat (*Triticum aestivum* L.) cultivars

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(With 2 figures)

Summary

Anther cultures have been performed to produce pollen plants from all members of the Austrian national list of winter wheat (*Triticum aestivum* L.) cultivars. In two of 31 cultivars no plantlets were produced and three produced only albinos. More than half of the cultivars, including high-baking quality and high-yielding cultivars, produced satisfactory numbers of green plants (more than one per 100 anthers). The mean percentage of green pollen plants was 9.4 % (11 cultivars in the first year), and 3.6 % (31 cultivars in the second year). A number of cultivars that gave low response in other studies, gave good yields of pollen plants in the present.

Key-words: wheat, anther culture, regeneration rate, varietal differences.

Antherenkulturtauglichkeit der österreichischen Winterweizensorten

Zusammenfassung

Von allen Winterweizen der österreichischen Sortenliste 1989 wurden Antherenkulturen zur Produktion von Pflanzen aus den Pollen angelegt. Von insgesamt 31 Sorten bildeten nur zwei Sorten keine Pflanzen, weitere drei bildeten nur Albinos. Mehr als die Hälfte der Sorten, sowohl Hochqualitäts- als auch Hochleistungssorten, bildeten eine ausreichende Anzahl grüner Pflanzen (mehr als eine pro 100 Antheren). Die durchschnittliche Ausbeute an grünen Pflanzen war im ersten Jahr 9,4 % (11 Sorten) und 3,6 % im zweiten Jahr (31 Sorten). Eine Reihe von Sorten, die in anderen Untersuchungen geringe Ausbeuten an grünen Pflanzen gebracht hatten, ergaben in diesen Versuchen gute Ausbeuten.

Schlüsselworte: Weizen, Antherenkultur, Regenerationsrate, Sortenunterschiede.

1. Introduction

Development of new cultivars in self-pollinating crops usually consists of three steps: creation of variability by crossing, homozygotization by several generations of selfing, and selection of desirable recombinants.

The in vitro anther culture technique produces haploid plants directly from the pollen grain. These plantlets are either treated with colchicine or chromosome doubling occurs spontaneously. Homozygous plants can therefore be obtained in one generation. This fact offers great potential in shortening the time required from crossing to the release of new cultivars (HENRY and DE BUYSER 1990).

In contrast to barley breeding, the use of anther culture in wheat breeding is still not routine practice despite great progress in anther culture technology. Two reasons make breeders reluctant to invest money and time in this technique. The one is the high variability of the frequency of dihaploids, and the other are fears that anther culture may introduce somaclonal variation into the breeding material of this polyploid crop plant. Comparison of published data reveal great variability in anther culture response when the same cultivars were used (HUANG 1987, OUYANG et al. 1987). Although a general technique to overcome genotypic variability would be desirable, the question whether the cultivars and the breeding material used in a specific breeding program produce dihaploid plants in sufficient numbers, remains of primal interest.

Little data exist until now about the anther culture response of Austrian wheat varieties. We undertook an anther culture response screening of registered cultivars from the Austrian national list of winter wheats including some foreign, mostly German, cultivars.

2. Material and methods

2.1 Experimental material

The cultivars used in 1989 represent the most important high-quality cultivars of winter wheat grown in Austria as well as five other high-yielding varieties. All members from the national list of registered winter wheat cultivars were tested for anther culture response in 1990. The cultivars were grown in the field in 1989 and 1990.

For all experiments, the spikes were cut before ear emergence and watered in a pail for two weeks in a cold room at 4° C under artificial illumination during the day (12 h).

The developmental stage was determined by acetocarmine staining of the microspores taken from the largest spikelet in the middle of the spike. Only the late uninucleate stage according to HE and OUYANG (1984) was used.

2.2 Embryo induction

In 1989, spikes in the proper stage were wrapped in wide mesh cotton cloth, disinfected with 0.1 % mercury chloride for eight min. and were then washed four times with sterile distilled water (CHUANG et al. 1978). In 1990, the tillers were sprayed with 70 % ethanol, and the spikes were removed under aseptic condition. As an induction medium, the potato-2-medium (CHUANG et al. 1978) with the following changes was used: 1500 mg/l KNO_3 instead of 1000 mg/l (FENG and OUYANG 1988), addition of 1 mM glutamine (HENRY and DE BUYSER 1981), 9 % commercial sugar, 0.6 % Difco agar Noble (medium P). All ingredients were mixed and autoclaved except glutamine which was filter-sterilized and added to the medium when it had cooled down to about 40° C.

A second induction medium was prepared using the same ingredients as for the medium P but with the agar replaced by agarose (type 15 from SIGMA), and sucrose autoclaved in the presence of 2 % activated charcoal (ZHANG et al. 1987). The charcoal was removed by filtration before medium preparation. All components except the potato extract and the agarose were filter-sterilized (medium S).

In the 1989 experiment, the anthers from one vertical half spike were cultured on medium P, the other half on medium S in the two halves of a divided petri dish. For the cultivar experiment in 1990, 50 anthers were taken from ten spikes per cultivar and cultivated on medium P in 50 mm petri dishes.

For embryo induction, the cultures were kept at 28° C and at high humidity in the dark for six weeks.

2.3 Plantlet regeneration

For plantlet regeneration, the medium 190-2 was used (He and Ouyang 1984). Regeneration was carried out at 24° C in the light (2 to 4 kLux) at a day length of 16 h.

For further growth, the plantlets were transferred to a simple medium containing only the minerals of the regeneration medium and 1.5 % sucrose. The cultures were kept in the same conditions as for plantlet regeneration. Vernalisation of the regenerated green plants was carried out in vitro at 4° C, at a day length of 10 h. After eight to ten weeks, the regenerants were planted into pots (8 × 8 cm).

2.4 Statistics

For media comparison, the Wilcoxon Signed Ranks test was used omitting cases where both media performed in the same manner. Prior to analysis of variance, data from anther cultures were ln transformed. One spike represents one replication in 1990 (1989: each half spike is one replication).

Analysis of variance was not performed when Bartlett's test detected inhomogeneous variances. These were caused by remarkable differences among individual spikes within a genotype and even more between the level of response among different cultivars.

3. Results

To study the effect of the induction medium on pollen plant production, a comparison of two media (media S and P) was performed. In the Wilcoxon Signed Ranks test that includes data from all cultivars of the 1989 experiment, the number of spikes performing better on one of the two media was compared (table 1).

Table 1

Comparison of medium P and S with anthers from 12 cultivars (1989)

trait	number of spikes performing better on medium P and average rank ( )	number of spikes performing better on medium S and average rank ( )	probability*
1. Responding anthers per 100 plated anthers	93 (90.8)	77 (78.7)	0.061+
2. Embryos per 100 responding anthers	87 (74.4)	62 (74.1)	0.060+
3. Embryos per 100 plated anthers	90 (86.8)	73 (76.1)	0.062+
4. Green plants per 100 plated anthers	63 (63.2)	51 (50.3)	0.044*
— without cultivars, Expert, Ferdinand and Julius:	32 (34.9)	33 (31.0)	0.751
5. Albino plants per 100 plated anthers	39 (36.6)	41 (44.2)	0.352

* probability for equality (Wilcoxon Signed Ranks test)

The anthers of the two halves of the same spike were cultured on two media in a divided Petri dish. This experimental design eliminated both, spike effects and microclimate effects as far as possible.

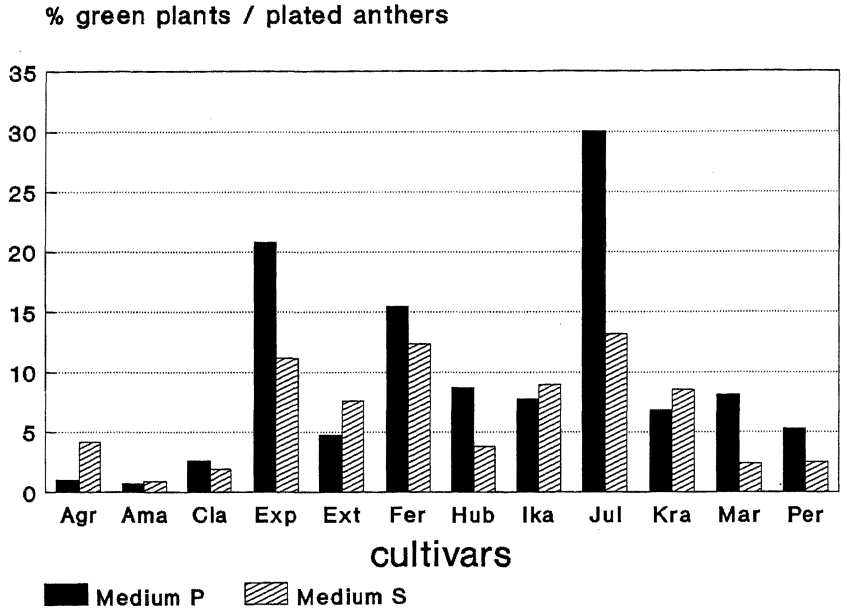

Fig. 1: Green plantlet yield from 12 cultivars on two induction media. Only three letters of the cultivar names are shown (see table 2 for complete names)

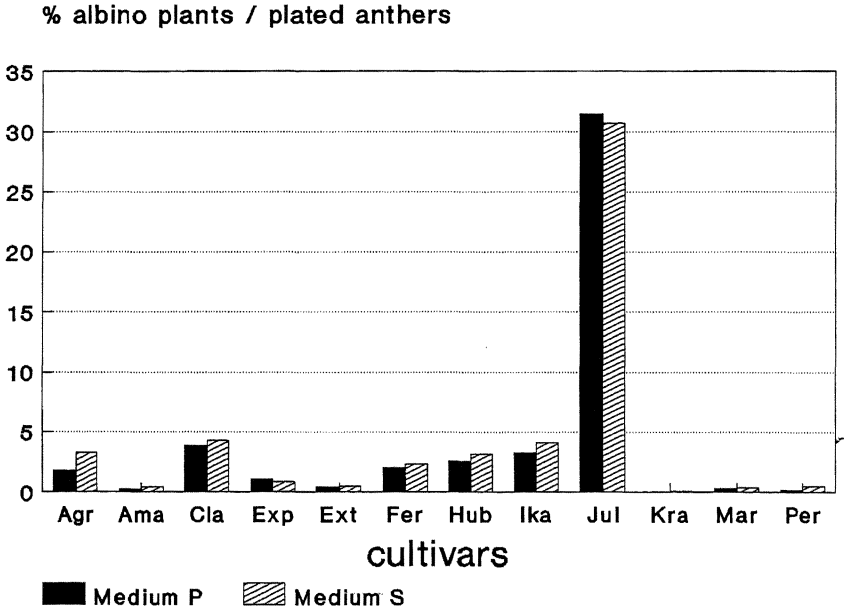

Fig. 2: Albino plant frequency of 12 cultivars on two induction media. Only three letters of the cultivar names are shown (see table 2 for complete names)

Embryo induction (traits 1 to 3 in table 1) was affected differently by the two media with 94 % significance. Green plantlet yield based on plated anthers (trait 4) was significantly higher on medium P (fig. 1 and 2). This was particularly obvious with the cultivars Julius and Expert.

All of the 12 cultivars tested in 1989 produced plantlets (table 2). Of the 31 cultivars tested in 1990, 29 produced plantlets and 26 produced green plants. The low number of anthers plated (400 to 500 anthers per genotype) may have precluded a positive response in the other cultivars.

Table 2

Anther culture response of the winter wheat cultivars (medium P) from the national list of cultivars (minimum number of anthers plated per genotype in 1989: 363, in 1990: 400). All traits are based on the percentage of plated anthers

Cultivar	% responding anthers		% embryos		% green plants		% albino plants	
	1989	1990	1989	1990	1989	1990	1989	1990
Adam		0.0		0.0		0.0		0.0
Agron*	3.8	1.2	7.7	1.2	1.0	0.2	1.8	0.0
Amadeus*	3.7	1.2	4.8	1.8	0.7	0.8	0.2	0.0
Aquila		2.0		2.8		0.8		0.0
Artus		2.6		4.6		0.0		0.6
Brokat		5.4		7.4		2.4		1.2
Claudius	19.4	12.0	38.4	23.6	2.6	1.2	3.9	3.6
Compact		2.0		2.0		0.0		0.2
Citadel		1.5		3.3		0.2		0.0
Disponent		21.4		56.8		19.8		0.8
Donau		9.0		19.8		1.0		2.4
Erla Kolben		2.2		3.6		0.2		0.0
Expert*	12.8	5.4	34.8	16.4	20.9	10.2	1.1	1.0
Extrem*	6.7	10.6	17.7	36.4	4.8	15.4	0.4	1.0
Famulus		0.6		0.6		0.0		0.2
Ferdinand*	14.9	17.2	31.7	48.0	15.5	22.2	2.0	2.6
Heiduck		9.2		11.6		1.2		0.4
Herzog		9.2		17.8		5.0		1.0
Hubertus	14.6	12.0	34.4	15.2	8.8	0.6	2.5	0.6
Ikarus	13.4	7.0	26.0	10.8	7.8	2.6	3.3	1.4
Julius	28.6	18.2	95.9	44.0	30.0	12.3	31.5	17.4
Karat		8.0		18.4		0.2		0.2
Magnus		7.4		11.6		0.4		0.8
Martin*	3.7	4.4	10.6	8.9	8.1	5.0	0.3	0.2
Perlo*	6.1	3.3	10.1	6.0	5.3	2.0	0.0	0.2
Pokal		0.0		0.0		0.0		0.0
Regent		1.8		1.0		0.0		0.2
Sorbas		6.9		12.4		0.7		0.2
Rektor		6.4		9.4		0.6		1.0
Titus		6.8		8.8		1.2		3.0
Kraka**	8.6			17.4		6.8	0.0	
Mean	11.4	7.0	27.5	14.6	9.4	3.6	3.9	1.3
— 11 cultivars (orthogonal)	11.6	8.4	28.4	19.3	9.6	6.6	4.3	2.6

* high baking quality cultivars "Qualitätsweizen"
** not in the Austrian national list of cultivars

The percentage of responding anthers ranged from none in Adam and Pokal to 21.4 % in Disponent and 28.6 % in Julius (medium experiment). In 1989, Julius was outstanding with 96 embryos per 100 anthers plated (on medium P). The good performance of Julius was maintained in the percentage of regenerating

plantlets, 31.5 % being albinos and 30.0 % green plants. In 1990, Ferdinand produced the highest frequency of green plants (22.2 %), followed by Disponent (19.8 %) and Extrem (15.4 %). Julius gave less regenerated plants than in 1989 (12.3 % green and 17.4 % albino plantlets). The other cultivars responded equally or less well than in 1989.

Table 3

Analysis of variance over the orthogonal part of 1989 and 1990 (11 cultivars) after ln transformation: mean square and probability for equality

trait	year	cultivar	year * cultivar interaction
% responding anthers	10.229 0.000***	7.612 0.000***	1.034 0.000***
% green plants	3.787 0.028*	4.013 0.000***	0.731 0.488 n.s.
% albino plants	4.487 0.051+	5.217 0.000***	0.300 0.594 n.s.

Table 3 shows the results of an analysis of variance over the orthogonal part of the cultivar experiments in 1989 and 1990 (both medium P). Significant interaction between genotype and year could only be detected for the trait "responding anthers" but neither for green nor albino plantlet production. This means that for green plantlet formation, the better cultivars in the first year remained statistically the better ones also in the second year.

4. Discussion

The direct comparison of anthers from one spike in two halves of the same Petri dish allowed a most precise comparison of the medium effect on pollen plant formation in wheat anther cultures. Medium P turned out to be slightly better than medium S.

The differences in response to the two media were smaller than those found by ZHANG et al. (1987). This can be due to the use of different genotypes, to the addition of glutamine to both media — ZHANG et al. used glutamine only in medium S —, to the addition of more KNO_3, to the use of commercial sugar rather than purified sucrose, to a more purified agar and their combinations. The data indicate that pollen plant yield can still be increased by a different combination of the compounds of the media. The fact that in the cultivar Julius less green plantlets were formed on the regeneration medium when embryo induction had taken place on medium S shows that the processes affecting albino formation start early in pollen embryogenesis but become visible only later, i.e. after the formation of shoots. This is another example of how the induction medium can influence further development on the regeneration medium (FENG and OUYANG 1988).

Genotypic differences in anther culture response are a well-known phenomenon. Such differences were highly significant for the range of cultivars used in this study as well as for F_1 hybrids and the breeding populations from F_2 to F_4 (LÖSCHENBERGER and HEBERLE-BORS unpubl. results). Remarkable segregation in anther culture response was also detected among the spikes cut from sublines (descendants of individual spikes) in the F_3 and F_4 generation (LÖSCHENBERGER et al. 1991). It has to be taken into account that the subpopulations which respond best will be overrepresented in the breeding program.

The cultivars that were tested in both years performed in a comparable way indicating that well-responding genotypes give good results also when several experimental conditions are slightly different (growth conditions of the donor plants, sterilization procedure, selection of anthers, medium preparation, in vitro conditions). Out of 31 cultivars, 17 yielded more than 1 % green plants and were therefore judged to be well-responding.

When compared to data of other authors working with the same genotypes, the results of this study often showed higher values. MÜLLER et al. (1989) reported 0.6 % (per plated anthers) green plants for Ikarus, while we found 8.5 % in 1989 and 2.6 % in 1990. FOROUGHI-WEHR and ZELLER (1990) obtained about 0.5 % total regenerated plantlets with the cultivar Kraka, below 1 % embryogenic structures and no green plantlets with Sorbas, Heiduck and Artus. We obtained on similar induction media 19.4, 12.4, 11.6, and 4.6 % embryos with Kraka, Sorbas, Heiduck and Artus, respectively, and green plantlets with all of these cultivars. Reasons for these good yield may be slight differences in the medium composition, such as the elevated KNO_3 concentration and addition of glutamine as compared to MÜLLER et al. 1989 or different gelling agent as compared to FOROUGHI-WEHR and ZELLER 1990. Other factors may have contributed as well, such as the scrutiny of stage determination, sterilization procedure, environmental and climate conditions, etc. The present result with Kraka is comparable to the 21.6 % of embryos on the sophisticated liquid potato-2 medium with addition of Ficoll and maltose (FADEL and WENZEL 1990).

For breeding with dihaploids, it is of utmost importance to have an estimate of the anther culture response of the material obtained after the initial cross. Such an estimate depends on the combining ability for anther culture response of the parents of the respective cross. A general combining ability may be a general phenomenon (AGACHE et al. 1989, LAZAR et al. 1984, TUVESSON et al. 1989). Although only half of the cultivars tested showed a satisfactory anther culture response (i.e. higher than one green plantlet per 100 planted anthers), many combinations of the cultivars used should allow satisfactory yields of green pollen plants. This is demonstrated by the fact that all breeding populations from the collection used for anther culture produced green plantlets (LÖSCHENBERGER and HEBERLE-BORS unpubl. results). Looking up the anther culture response of the cultivars tested allows now to estimate about the anther culture response of a hybrid. In that sense the present work could contribute to promote the use of anther culture in winter wheat breeding programs.

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