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Significant improvement of androgenetic haploid and doubled haploid induction from wheat plants treated with a chemical hybridization agent

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Summary. A very significant improvement of the total yield of androgenetic green plants after anther culture is presented. The process involves treatment of the donor plants by spraying at different stages around the meiosis with a chemical hybridization agent (CHA) solution, fenridazon-potassium. When harvested at the normal uninucleate pollen grain stage, anthers have shown during in vitro culture very significant increases in embryo production. Compared to the control, we observed up to a 20-fold increase in the production. Moreover, when cultivated later, anthers still remained embryogenic. Therefore the process appears to be very efficient and to allow a broadening of the target period for androgenesis in vitro. The regeneration was not disturbed by the CHA treatment and, as shown in this paper, the technique seems to be applicable to a large range of genotypes. Cytological observations revealed both a low frequency of aneuploidy among the regenerated plants and peculiar features in the pollen grain walls after treatments; a triploid plant was observed. Hypotheses to explain the phenomenon are presented and related to previous observations on the effects of gametocide substances like ethrel, male sterility and pollen dimorphism on androgenesis.

Key words: Bread wheat – Androgenesis – Improvement of haploid production – Chemical hybridization agent – P-grains

Introduction

The potential effect of a gametocidal substance like ethrel (2-chloro ethyl phosphonic acid) on the mitotic apparatus of wheat pollen grains has been known for a long time (Bennett and Hugues 1972). This discovery of additional mitosis within pollen grains of plants treated with ethrel has raised the hope that this or similar substances might improve the haploid production in wheat and other cereals. Unfortunately, the authors were not successful in the later steps, i.e. the embryo and plantlet production. Everybody who tried to repeat the work or go further never obtained a clear improvement; at most, a slight improvement of embryo production has been described in rice and wheat (Wang et al. 1974; Bajaj 1977; Hu et al. 1978). Nevertheless, the positive relationship between cytoplasmically induced male sterility and the embryogenic ability of wheat anthers has been observed for isogenic lines on cytoplasms of *Triticum aestivum* and *Triticum timopheevi* with a clear advantage to the sterile lines (Picard et al. 1978). More recently, two F_2 lines of wheat possessing "timopheevi" cytoplasm were observed to be more embryogenic than other lines (Heberle-Bors and Odenbach 1985). The latter have clearly correlated this with their observations on tobacco pollen grains, i.e. the microspores suitable for haploid production are the so called "P-grains" or functionally sterile pollen grains (Heberle-Bors 1985). They showed that P-grains are also obtained with other cytoplasms of the *Triticum* series such as "*caudata*", "*speltoïdes*". Even though it is difficult to use the criterium of P-grains frequency, because it can be applied only after the date of anther harvest, it remains a good a posteriori proof. For example, in anthers of some androgenetic doubled haploid lines, we observed these P-grains exhibiting additional mitosis with up to six nuclei in the same pollen grain (de Buyser and Picard 1975). These lines have been tested also in anther culture and found to be better embryogenic lines than their parents (Picard and de Buyser 1977). All the above observations are in good

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agreement and Heberle-Bors (1985) postulates very clearly that all the conditions shifting the sex balance – such as pollen dimorphism, male sterility, feminizing substances –, are likely to induce a better haploid production from anther culture. The results that we describe hereafter provide a good experimental check of these concepts. While researching the effects of CHA treatments on embryo development in wheat, we attempted anther and ovary cultures of the treated plants. Ovary cultures had no success. Consequently this paper reports only on the anther culture which gave very valuable improvements in androgenetic yields, opening a new perspective for haploid production and breeding programmes.

Materials and methods

Experiment 1

Plants of an F_1 hybrid between two soft wheat varieties (cv. "Fielder" × cv. "Chinese Spring" ditelosomic 3D) were used. The ditelosomic lines was chosen as a cytological marker in our previous experiments. A total of 30 F_1 plants were grown in a regulated and illuminated (16 h/day) glass green house, following 3 weeks of artificial vernalization at 5 °C. All except six plants were treated by spraying with two doses of a CHA solution at one or three growth stages according to Feekes' Scale (FS). Six plants per dose × stage were treated by manual spraying with a CHA water solution containing 0.03% Triton X-100. The combinations are:

Dose 1: 0.75 kg ha^{-1}; three stages were used: t1, before meiosis, FS = 8.2 to 9.0; t2, during meiosis, FS = 9.1 to 9.3; t3, after meiosis, FS = 9.3 to 9.5.

Dose 2: 1 kg ha^{-1} at only one FS stage, that is before meiosis, FS = 8.2 to 9.0.

The CHA was given to us by the ROHM and HAAS Company. It is RH 0007, commonly known as fenridazon potassium (McRae 1985), and induces 95%–100% male sterility in wheat. It was used at a concentration of 1.05 g l^{-1} for dose 1 and 1.4 g l^{-1} for dose 2. For anther culture the spikes were removed at two stages:

1. at the usual stage for in vitro androgenesis, i.e. at the mid to late boot stage (FS = 9.3 to 10) or
2. at a later stage than usual, i.e. when the upper spikelets have completly emerged (FS = 10.1 to 10.3). The spikes of the two sets are then conserved in water at 3 °C, for 2–8 days, and the anthers then plated on Miller's medium with potatoe extract of 100 g tubers l^{-1} added according to Chuang et al. (1978). The original positions of the anthers in their spikes were identified in the petri dishes. When visible, the embryo-like structures were counted at different steps during the culture; they were then transferred onto R9 regeneration medium (Picard and de Buyser 1977).

Experiment 2

The second experiment was performed on a wheat population obtained in our laboratory after three successive crosses through a circular cross scheme with the following ten parent lines chosen for their agronomic (A) or embryogenic (E) values: "Fidel", "Pernel", "Arminda", "Festival", "Camp-Remy", "Galahad", "Frandoc", "Rescler", as "A" lines and 022 D (CC. Benoist line) and a doubled haploid line, produced by us, as "E" lines. Gallais (1983) has suggested that the circular cross is the best way to combine alleles originating from different parents. A sample of this population, grown in the same greenhouse conditions, was used in experiment 2 to test the effect of CHA on androgenesis in virto on a broader genetic basis. Among the 19 final different crosses, represented each by three plants, we treated one plant per cross at 1 kg.ha^{-1} and FS stage 8.0 to 9.2. The two remaining ones were used as controls. Two abbreviations are used in the text and the Tables: %EMB: percentage of observed embryos in relation to the number of cultivated anthers; %EMBSP: percentage of the embryogenic spikes giving at least one embryo in one experimental unit.

Results

Experiment 1

General results. This experiment used Fielder × Chinese Spring ditelo 3D. Table 1 summarizes the results obtained with anthers collected at two phenotypic stages of the spikes on plants treated by the CHA solution (see "Materials and methods") at different stages and doses, and compared to untreated plants. This demonstrates very clearly that treatment with a CHA induced an important improvement in the total yield of embryos from anther culture. For instance, with a dose of 1 kg ha^{-1} the %EMB reached 27.98% (431 embryos produced from 1,540 anthers), which is 20 times the control (%EMB 1.36%, 20 embryos for 1,470 anthers). If we now compare the total yield obtained for all treated plants with spikes sampled at the usual stage, we observe an overall 10-fold increase compared to the controls. This is also true for the performance of pollen from spikes harvested later. Indeed, while %EMB observed in untreated anthers decreased to near zero (0.08%), it remained rather good at up to 14.97%, for treated anthers. This suggests that the period for harvesting spikes on the mother plants is much longer for treated than for untreated plants. As we applied the 1 kg dose of CHA at only one stage, we can only underline that, in this case, it is better than the lower dose (0.75 kg ha^{-1}). Yet the 0.75 kg ha^{-1} dose was tested at three stages – before, during and after meiosis – and the pollen response was very quick. Better %EMBs per spike were observed for treatment after meiosis, with two maxima of 72.8% and 114%. It seems, therefore, that the action of the CHA solution on pollen was almost immediate. If we look at the %EMBSP, the period of meiosis is perhaps a little less fovourable than the others, two yielding the two minima observed for this parameter.

Results expressed as a function of spike rank. The response from the sets of microspores from successive

Table 1. Results of androgenesis in vitro applied to material harvested from wheat plant treated with two doses and different stages by a chemical hybridization agent (CHA) solution and from untreated plant. For abbreviations and detailed stages, see "Materials and methods"

		Plants used	Spikes used	Cultivated anthers	Observed embryos	%EMB	%EMBSP	%EMB max per spike	%EMB min per spike
		Results from spikes harvested at the usual stage: Feekes' scale stage 9.3 to 9.5							
Control		6	21	1,470	20	1.36	42.9	5.7	0
0.75 kg ha^{-1}	Before meiosis d_1t_1	5	21	1,470	259	17.61	71.4	61.4	0
	During meiosis d_1t_2	8	28	1,916	97	5.06	32.0	37.1	0
	After meiosis d_1t_3	5	20	1,400	160	11.43	45.0	72.8	0
1 kg ha^{-1}	Before meiosis d_2t_1	6	22	1,540	431	27.98	100.0	65.7	7.1
Total for treated plants		24	91	6,326	947	14.97	62.1	72.8	7.1
		Results from spikes harvested at a later stage: Feekes' scale stage 10.1 to 10.3							
Control		6	19	1,290	1	0.08	5.2	1.4	0
0.75 kg ha^{-1}	Before meiosis d_1t_1	5	14	980	57	5.80	71.4	18.6	0
	During meiosis d_1t_2	8	25	1,704	118	6.92	28.0	94.3	0
	After meiosis d_1t_3	5	16	1,064	159	14.90	43.7	114.0	0
1 kg ha^{-1}	Before meiosis d_2t_1	6	20	1,313	170	12.94	80.0	97.1	0
Total for treated plants		24	75	5,061	504	9.96	55.77	114.0	0

spikes depends on the rank of these spikes and on the dose of CHA (Fig. 1). Figure 1 represents results obtained at the usual stage of harvesting the spikes. If we compare d_1t_1 (▲–▲) and d_2t_1 (○–○), anthers originating from plants treated at the same stage but not with the same dose, the anthers remained highly embryogenic through the first six spikes at 1 kg ha^{-1} (d_2), whereas at 0.75 kg the %EMB decreased beyond the second spike. Moreover, sufficient delay between treatment and harvest is required for efficiency over all the successive spikes, as is obvious when comparing the 3 stages for dose 1. We note an additional problem with non-receptivity for the meiosis stage. Results obtained from spikes harvested later (not shown in Fig. 1) are different, although also variable, and indicate that better responses were obtained for stage t_3.

Effect of the rank of the spikelets. Figure 2 displays the pooled means of the embryos obtained for the two series of harvests, expressed as %EMB in relation to the original position of the anthers within the spikes, for the different doses and stages. The control is also shown. The general superiority of the treated microspores over the untreated shows up very clearly, whatever the dose or stage. The best results, for example for

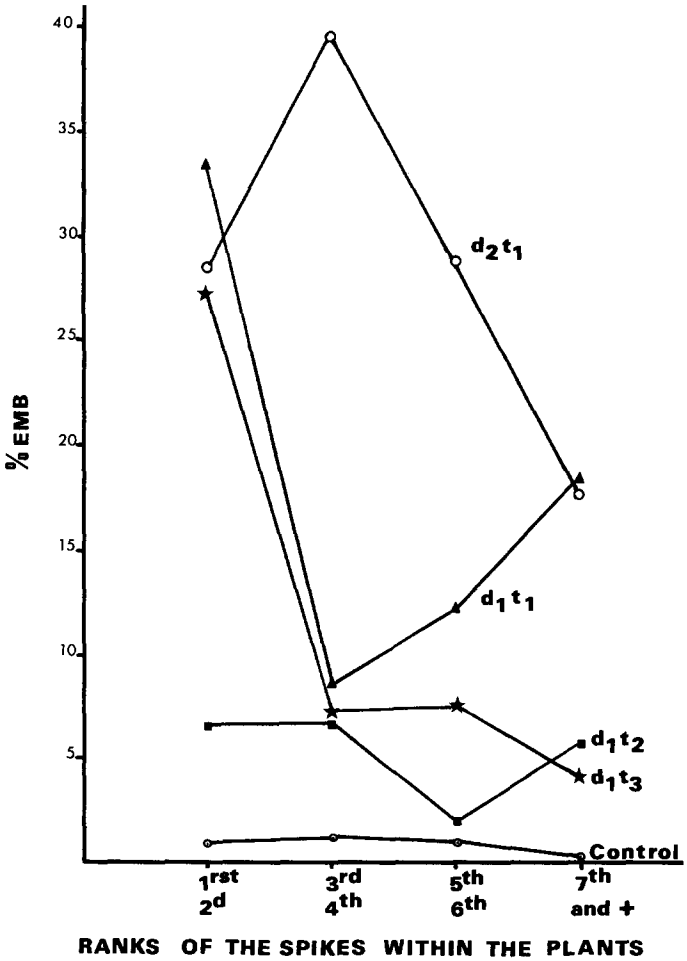

Fig. 1. Observed %EMB in relation to spike order; spikes collected at the normal stage from CHA treated plants or from untreated plants. ▲ d_1t_1; ○ d_2t_1; ■ d_1t_2; ✱ d_1t_3; ⊙ control

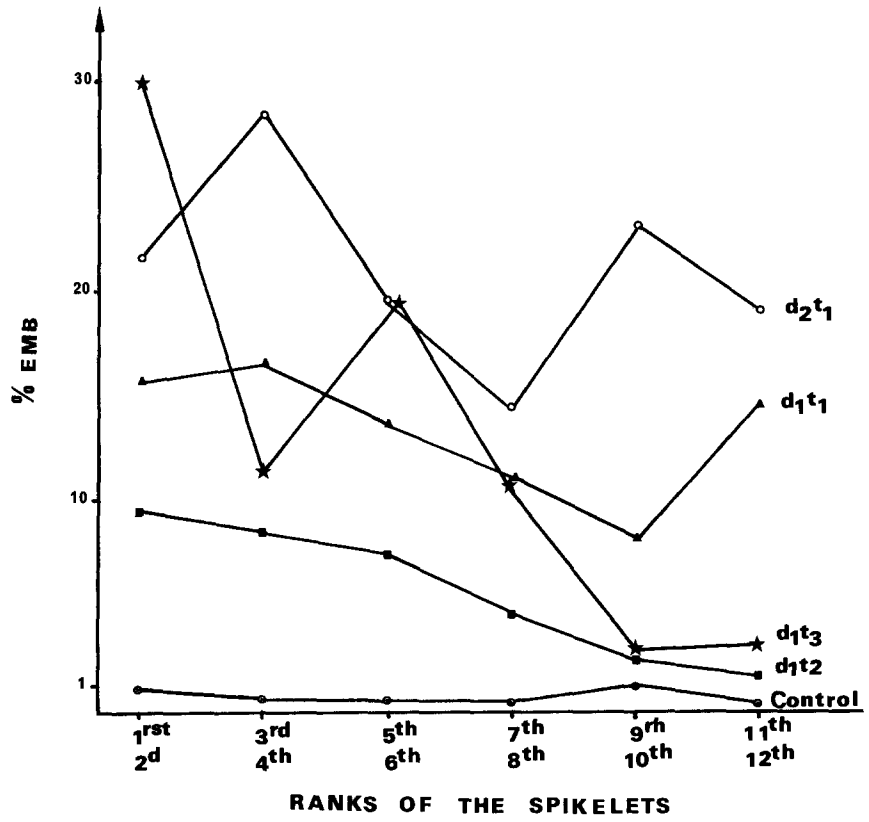

Fig. 2. Percentages of embryos (%EMB from different parts of the spikes) from the lower (left) to the upper one (right) in various cases: control, without treatment (⊙—⊙), each point represent 460 anthers; CHA treated material at d_1t_1 (▲—▲), d_1t_2 (■—■) and d_1t_3 (★—★) with 396, 622 and 410 anthers per point respectively and d_2t_1 (⊙—⊙) with 475 anthers per point

the first to the fourth spikelets at d_2t_1 and d_1t_1, showed a 60-fold increase in the %EMB. The androgenetic ability of the anthers which originated from plants treated at t_1, that is before meiosis along the spikes is also relatively stable. One can observe a decrease in this embryogenic capacity towards the top of the spike for stages t_2 and t_3. The chemical may be distributed gradually along the spikes and it therefore first reaches the microspores of their lower parts. After a sufficiently long time, the gradient more or less disappears. CHA may also have a more toxic effect on the upper part of spikes treated at stages t_2 or t_3. Thus this kind of treatment may be able to overcome the position effect that we demonstrated in previous studies (Picard and de Buyser 1975, 1977).

Kinetics of embryo emergence from the anthers. The period and the kinetics of embryo emergence are perceptibly modified by the CHA treatment. The first embryos from treated anthers appeared 8 days sooner than those from untreated anthers but were also produced up to the 30th day, as in the control. The production per anther and per day was always much higher for the treated anthers (Fig. 3). After the first embryo emergence, the increasing production phase was 8 days in treated but only 2 days in untreated material (Fig. 4). Moreover, during this phase, the rate of embryo production in the control was twice that of the treated plants. After picking, we observed a slow decrease in production for the treated anthers while, by contrast the control showed a rapid decrease in production. Finally between days 20 and 30 of culture, the production of embryos decreased regularly in the same way in both cases.

In comparison to what is usually observed in wheat anther culture, the treatment with CHA seems to have desynchronized the microspores. Moreover, the embryos could be produced in vitro much more quickly than has been commonly supposed.

Cytological observations of the pollen. Several anthers were fixed in ethanol and acetic acid (3:1) at the beginning of the cultures for observation. Their spikes were classified into four types according to the number of embryos they produced during anther culture. Numbers of dead and live pollen grains were counted from a set of 8–13 anthers for each group. Variance analysis showed that the group of non-embryogenic,

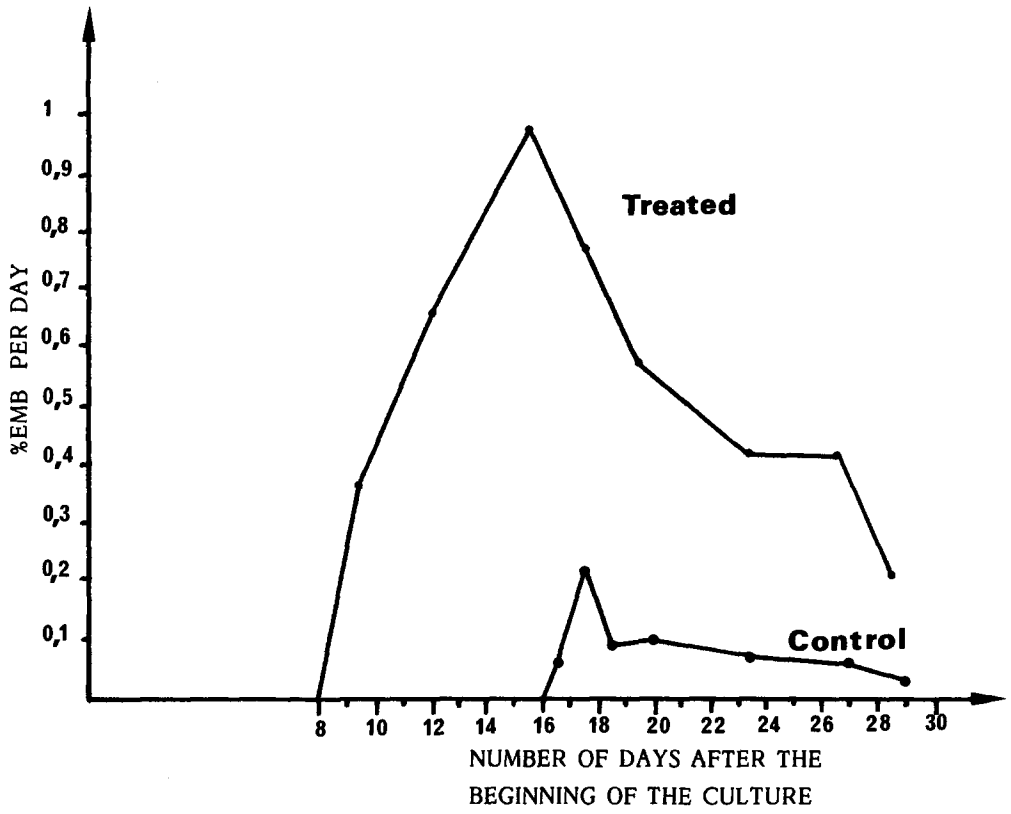

Fig. 3. Embryo appearence from anthers harvested from CHA treated and untreated plants relative to day of harvest

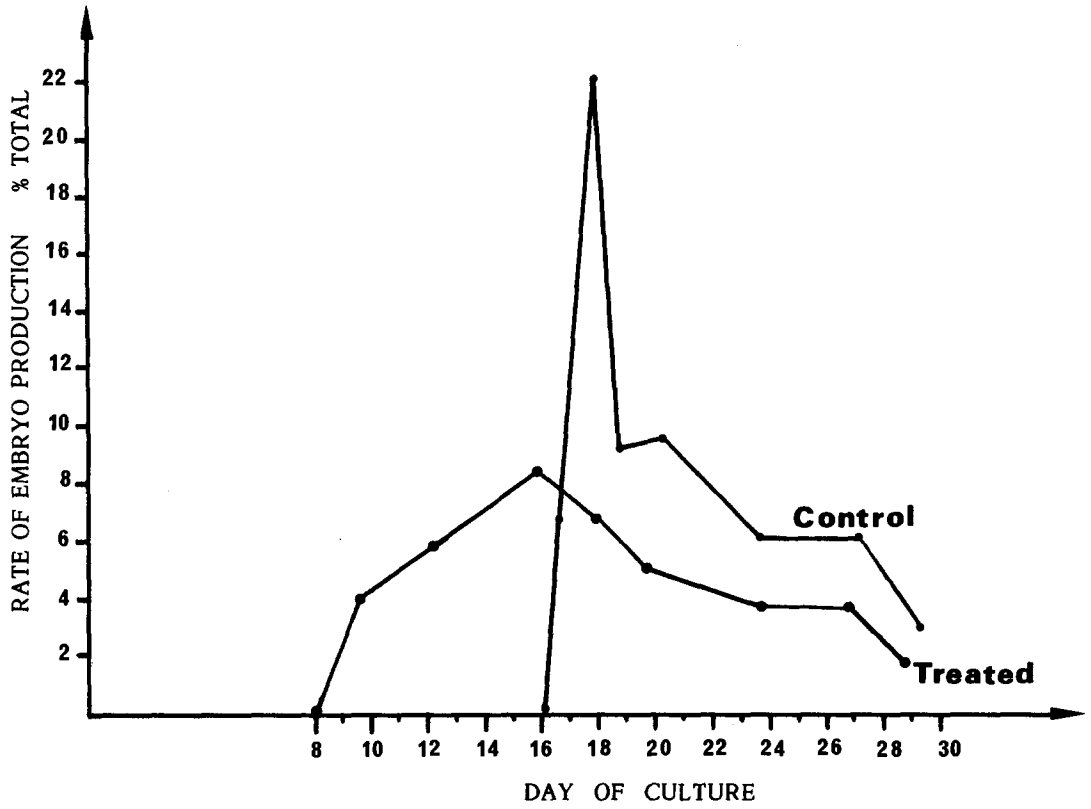

Fig. 4. Rate of embryo appearance in CHA treated and control material relative to day of harvest in percent of the total for each case

Table 2. Comparative study of the numbers of living and dead pollen grains observed in the anthers of different classes of spikes: no. of untreated and treated (*1*) spikes having produced. (*2*) no embryos; (*3*) 10 embryos; (*4*) more than 20 embryos. Non-significantly different means or variances are denoted by the same letter ($P=0.05$)

	Types of spikes			
	Untreated spikes control (1)	Spikes producing no embryos (2)	Spikes producing 10 embryos (3)	Spikes producing more than 20 embryos (4)
No. of anthers observed	8	9	10	13
No. of living pollen grains ($\bar{x}$) per anther	1,908 (a)	1,100 –	553 (bc)	820 (c)
No. of dead pollen grains ($\bar{x}$) per anther	391	205	377	374
Total	2,299	1,305	930	1,194
σ^2 living pollen grain	119,521 (a)	1,037,041 (b)	501,391 (a)	528,278 (a)

Table 3. Observed regeneration rates (green plantlets) for embryos harvested from CHA treated material Fielder × Chinese Spring, at different doses × stage and from same genetic material untreated (see "Materials and methods")

	Doses × stages					
	d_1t_1	d_1t_2	d_1t_3	d_2t_1	Treated total	Untreated total
Embryos	2	88	109	162	361	6
Green plantlets	1	15	15	15	46	2
%	(50%)[a]	17%	13.8%	9.2%	13%	(33%)[a]

[a] No. of translated embryos for valuable comparison too low

treated spikes displayed a higher variability ($P=0.05$) than the 3 other groups (Table 2). The observed number of live pollen grains in this type of spike was either nearly zero, as the CHA effect had been too strong, or more or less equal to the control number, as the pollen grains were not affected by the CHA. In the two other classes of treated spikes, the means of live pollen grains were significantly different from those of the control, but not from each other. In these two classes, the number of viable pollen grains was 30% to 43% of the control and the variation was not correlated with the number of produced embryos ($r=0.1$). Thus the high production of embryos could not be explained only by a decrease in pollen competition, suggested by the decrease in living pollen grains.

The morphological study of the pollen grains from treated plants and especially of their cell walls, revealed a high proportion of deficient pollen grains, up to 100%. Those abnormal pollen grains exhibited shrivelled cell-walls, probably exine-deficient with non-stainable cytoplasms. Compared to the pollen grains coming from control plant, they were also lacking starch grains. Additionally we have noted a higher nucleolar activity.

Regeneration and chromosome counting. In order to check the ability of the embryos to regenerate green plantlets after CHA treatment, embryos (361 out of a total 1,451) were transferred to an R9 medium. Unfortunately, a mistake in the composition of the medium made it necessary to transfer them a second time on to a new fresh medium 2 weeks later. This could have decreased the rates of regeneration in Table 3. Nevertheless the regeneration percentage is, on average, as high as 13%, with 46 green plantlets obtained from 361 embryos transplanted twice and this is not low. In fact, it reached 17% in d_1t_2. Thus it could be assumed that the organogenesis had not been affected by the CHA itself.

Feulgen's technique was used for the chromosome count. Of 32 counted plantlets from treated plants, we found 17 true haploids (53.1%), 7 true diploids (21.8%), 3 haploid-diploid (9.4%), 1 mixoploid (3.1%) and 4 with a higher cromosomic number than the diploid (12.5%). Additionally, a case of triploidy was observed in another set, the first in wheat after anther culture. Nevertheless, with approximately 75% true haploids or diploids plantlets produced, CHA does not seem to

Table 4. In vitro androgenesis responses of a sample of 9 F_1 chosen for their particular behaviour and of the whole population (19 F_1) after CHA treatment, TR (treated) – Without treatment, UNT (untreated)

		Plants used	Spikes used	Anthers cultivated	Embryos observed	%EMB	Range	%EMBSP	Transfered embryos	Green plants	Albinos plants	Green plants/ embryos %	Green plants/ anther %
POP F_1-1	UNT	2	14	1,008	7	0.69	0– 2.78	42.8	7	0	0	0	0
	TR	1	13	936	26	2.78*	0–18.06	38.4	26	5	1	19.2	0.53
POP F_1-2	UNT	2	15	1,080	4	0.37	0– 2.78	20	4	2	0	50	0.19
	TR	1	10	720	19	2.64*	0–23.61	30	18	5	1	27.7	0.69
POP F_1-3	UNT	2	20	1,440	28	1.94	0–15.28	40	24	3	0	12.5	0.22
	TR	1	11	792	16	2.02 NS	0– 6.94	72.7	12	3	2	25.0	0.46
POP F_1-4	UNT	2	18	1,296	10	0.77	0– 5.5	27.7	9	1	0	11.1	0.08
	TR	1	12	864	35	4.05**	0–11.1	75	31	5	2	16.1	0.63
POP F_1-8	UNT	2	13	936	0	0	0	0	0	0	0	0	0
	TR	1	7	504	9	1.79**	0– 5.56	57.1	9	3	3	33.3	0.60
POP F_1-10	UNT	2	14	1,008	21	2.08**	0–22.2	28.5	21	0	0	0	0
	TR	1	8	576	1	0.17	0– 1.3	12.5	1	0	0	0	0
POP F_1-11	UNT	2	24	1,728	21	1.22	0– 6.94	33.3	20	2	1	10	0.12
	TR	1	10	720	112	15.5**	0–36.11	80	102	27	5	26.4	3.75
POP F_1-14	UNT	2	11	792	0	0	0	0	0	0	0	0	0
	TR	1	16	1,152	61	5.30**	0–22.2	62.5	56	10	1	17.8	0.93
POP F_1-18	UNT	2	6	432	2	0.46	0– 2.7	16.6	2	0	0	0	0
	TR	1	7	504	16	3.17**	0–11.1	42.8	16	5	5	31.2	0.99
Total for the 19 F_1	UNT	38	299	21,528	174	0.81	0–22.2	23.4	159	15	7	9.43	0.07
	TR	19	177	12,744	478	3.75**	0–36.11	53.6*	434	84	21	19.35*	0.69**

upset the chromosome number of the regenerated plants too much and does seem to be useful. Another observation that proves the correct origin of the diploid plantlets is the presence or absence of the telosomic chromosome pair 3D in the diploid metaphases. The process of meiosis may not have been perturbed by the CHA and the spontaneous DH may have been derived from an early endoreduplication by endomitosis during the in vitro culture, as demonstrated in wheat by Raquin et al. (1982).

Experiment 2

This experiment involved CHA treatment of a wheat population. As has been already emphasized in Materials and methods, we tried to immediately apply this discovery to a larger set of genotypes, using a population of F_1' genotypes created by circular crosses. Table 4 gives the detailed results for 9 typical cases among the 19 tested F_1 crosses for untreated and treated plants as well as the results over all. We, mainly, observed that treated anthers yielded significantly more than untreated (14 cases), with only one exception (see POP F_2-10, Table 4). We observed an increase of up to 13-fold in %EMB and up to 30-fold for percentage of green plantlets in the treated material. We found 12 instances of significant superiority for %EMBSP in treated spikes. From treated plants we obtained androgenetic green plantlets for 14 out of 19 F_1', and the yield reached or surpassed 1 plant per 100 anthers in 4 F_1's. For untreated material, only 6 out of 19 F_1's yielded at least one green plantlet with a maximum of 2–3 plantlets for 1,000 anthers. The percentage of regenerated albinos plantlets decreased in treated material in comparison with the control.

The results indicate that the CHA treatment has induced obvious improvements for %EMB (5 fold), %EMBSP (2 fold), regeneration rate (2 fold) and finally total yield (nearly 10-fold). All these observations confirm the previous ones over a large spectrum of genotypes and underline the remarkable efficiency of this treatment, since only one plant for each genotype was treated in the present experiment.

Discussion

Firstly, it does not seem necessary to stress that the above study represents a great improvement in the haploid technology in wheat. In a recent communication, Schmid and Keller (1986), using another CHA, also described very similar and convergent results in wheat androgenesis. Their study and our own are the first instances of substantial progress in all parameters of a cereal anther culture. The suggestion of several authors (Bennett and Hugues 1972; Bajaj 1977; Heberle-Bors 1985) that any substance altering the sex balance could enhance haploid production has now been clearly demonstrated. It is therefore likely that gametocide or CHA could be a third method in addition to the physiological or genotypic ones for mass production of haploids in cereals. Nevertheless, let us underline that we did not surpass the best results which have been obtained, here and there, for particular wheat lines or hybrids (Ouyang et al. 1983; Hu Han 1986; Snape et al. 1986; Picard 1984) which were higher or equal to those of the present observations. For instance, both genetic and physiological improvements have been succesfully made in potato haploid production (Uhrig 1985). But CHA technology: (1) gives a 5- to 20-fold increase of haploid and homozygous plant production for any genotype, (2) enlarges the targed period for harvesting source material in the greenhouse, making this easier and (3) accelerates embryo development, reducing down to 8 days in culture. The quasi-elimination of the genotypic effects, as the above results tend to demonstrate, is certainly one of the most important features of this technique for breeding because the genetic drift it can cause, is eliminated at the same time. However, genotype × CHA or treatment × experiment interactions may be discovered.

We should not conclude from these results that the genetic method is useless. The transfer to breeding material of alleles responsible for high haploid production whose expression in vitro is heritable has been proposed by several authors (Fouroughi-Wher 1982; Wenzel and Uhrig 1981; Lazar et al. 1984; Picard 1984; genetic assimilation: Rives and Picard 1977). In the wheat population described and in a previous one, realized 2 weeks sooner with no CHA treatment, we obtained interesting results among the 19 crosses. The %EMB reached 10% in one cross and 1% green plantlet was obtained in another, whereas other crosses appeared to be non-embryogenic. The obvious effect of CHA is to standardise the responses of all the genotypes towards higher performances. However a study of the genotypic effect from the fundamental point of view is essential for understanding the genetic control of the male gamete behaviour in vitro, the origin of gametoclonal variation and the gene expression at the haploid level. Secondly, CHA substances are not actually available on the market. Thus the genetic method remains the one generally available for increasing haploid production.

It is needless to add that a complete cytogenetic study of the regenerated plants is required to ensure that CHA did not induce cryptic or dramatic DNA changes that would show up at the next meiosis or in the selfed progenies. Thus we need to remain cautious and to look for triploid plants, which are very rare in cereals. One advantage of this new technique is the relatively good rate of spontaneous diploid regenerated plants, approximately 30% of the total.

Hypotheses to explain the origin of all these phenomena are difficult to imagine because of the lack of published work about the effect of gametocides on pollen grains. We can follow Colhoun and Steer (1982), who conclude that the primary site of gametocide action in barley is the microspore rather that the tapetum. In fact we have emphasized in this paper the observations of exine deficient pollen grains with fine cell walls, which suggests that the structure is damaged and the number reduced. One can imagine that the reduction of the cell wall thickness allows a better exchange of nutrients and at the same time solves the common problem of dramatic proembryo degenerescence between the 8th and the 12th day of anther culture (Henry and de Buyser 1984). Ultrastructure analysis of modifications to the pollen wall remain to be done. One can add that these chemical agents are known as growth regulators, acting in this case synergistically on the microspore development in vitro, that is in turn more receptive to external elements.

Finally, for the plant breeder it is striking that the same chemical agent can induce a rapid obtention of homozygous lines and in due course, control their hybridization as hybrid parents.

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References

Bajaj YPS (1977) In vitro induction of haploids in wheat (*Triticum aestivum* L.). Crop Improv 4:54–64

Bennett MD, Hughes WG (1972) Additional mitosis in wheat pollen induced by Ethrel. Nature (London) 240:566–568

Chuang Ch Ch, Ouyang TW, Chia H, Cous S, Ching CK (1978) A set of potato media for wheat anther culture. Proc Symp Plant Tissue Culture. Science Press, Peking, pp 51–56

Colhoun CW, Steer MW (1982) Gametocide induction of male sterility: a review and observations on the site of action in the anther. Rev Cytol Biol Veg 5:283–302

De Buyser J, Picard E (1975) Observation de divisions suplémentaires dans les grains de pollen de plantes homozygotes de Blé tendre (*Triticum aestivum* L.) obtenues par androgénèse in vitro. CR Acad Sci Ser D 281:1153–1156

Foroughi-Wehr B, Friedt W, Wenzel G (1982) On the genetic improvement of androgenetic haploid formation in *Hordeum vulgare* L. Theor Appl Genet 62:233–239

Gallais A (1983) Etude de critères de sélection chez le maïs fourrage: héritabilités, corrélations génétiques et réponse attendue à la sélection Agronomie 3:751–760

Heberle-Bors E (1985) In vitro haploid formation from pollen: a critical review. Theor Appl Genet 71:361–374

Heberle-Bors E, Odenbach W (1985) In vitro pollen embryogenesis and cytoplasmic male sterility in *Triticum aestivum* L. Z Pflanzenzücht 95:14–22

Henry Y, de Buyser J, Guenegou T, Ory C (1984) Wheat microspore embryogenesis during in vitro anther culture. Theor Appl Genet 67:439–442

Hu H, Hsi TY, Tseng CC, Ouyang TW, Ching CK (1978) Application of anther culture to crop plants. In: Thorpe TA (ed) Frontiers of plants tissue culture 1978. Offset Print Serv, University of Galgary, Galgary, USA, pp 123–130

Hu Han (1986) Wheat: improvement through anther culture. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry 2 – crops I. Springer, Berlin Heidelberg New York, pp 55–72

Lazar MD, Baenziger PS, Schaeffer GW (1984) Combining abilities and heritability of callus formation and plantlet regeneration in wheat (*Triticum aestivum* L.) anther cultures. Theor Appl Genet 68:131–134

McRae HH (1985) Advances in Chemical Hybridization. In: Janick j (ed) Plant breeding reviews, vol 3. Purdue University, pp 169–192

Ouyang JW, Zhou SM, Jia SE (1983) The response of anther culture to culture temperature in *Triticum aestivum.* Theor Appl Genet 66:101–109

Picard E (1984) Contribution à l'étude de l'hérédité et de l'utilisation en sélection de l'haploïdisation par androgenèse in vitro chez une céréale autogame: *Triticum aestivum* L. Thesis, Université Paris XI, France

Picard E, de Buyser J (1975) Nouveaux résultats concernant la culture d'anthères in vitro de blé tendre (*Triticum aestivum* L.). Effets d'un choc thermique et de la position de l'anthère sur l'épi. CR Acad Sci, ser D 281:127–130

Picard E, de Buyser J (1977) High production of embryoids in anther culture of pollen derived homozygous spring wheats. Ann Amelior Plant 24:483–488

Picard E, de Buyser J, Henry Y (1978) Technique de production d'haploïdes de Blé par culture d'anthères in vitro. Le Sélectionneur Français 26:25–37

Raquin C, Amssa M, Henry Y, de Buyser J, Essad S (1982) Origine des plantes polyploïdes obtenues par culture D'anthères. Analyse cytophotométrique in situ et in vitro des microspores de Pétunia et de Blé. Z Pflanzenzücht 89:265–277

Rives M, Picard E (1977) A case of genetic assimilation: selection through androgenesis or parthenogenesis of haploid producing systems (an hypothesis). Ann Amelior Plant 27:489–491

Schmid J, Keller ER (1986) Improved androgenetic response in wheat (*Triticum aestivum*) as a result of gametocide application to anther donor plants. Abstracts of the 4th Int Cong Plant Tissue Cell Culture, 3–8 August, 1986. University of Minnesota, p 146

Snape JW, de Buyser J, Henry Y, Simpson E (1986) A comparison of Methods of Haploid Production in a Cross of Wheat, *Triticum aestivum* L. Z. Pflanzenzücht 96: 320–330

Uhrig H (1985) Genetic selection and liquid medium conditions improve the yield of androgenetic plants from diploid potatoes. Theor Appl Genet 71:455–460

Wang CC, Sun CS, Chu Z (1974) On the conditions for the induction of rice pollen plantlets and certain factors affecting the frequency of induction. Acta Bot Sin 16: 43–53

Wenzel G, Uhrig H (1981) Breeding for nematode and virus resistance in potato via anther culture. Theor Appl Genet 59:333–430