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Direct effect of colchicine on the microspore embryogenesis to produce dihaploid plants in wheat (*Triticum aestivum* L.)

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Summary. In the present experiment, different chromosome reduplication techniques were applied to microspore-originated *Triticum aestivum* L. (cv 'Ciano') haploids. In addition to the conventional treatment (whole plant exposure to colchicine solution), spontaneously redoubled haploids were also examined. As an experimental treatment, different concentrations (0.01, 0.02, 0.04%) of colchicine were added directly to the induction media. Colchicine did not affect the anther response or the plant regeneration capacity. The success and stability of genome redoubling was estimated on the basis of the fertility of the regenerated (R_0) plants and their progeny (R_1). Chromosome doubling produced by colchicine before the first microspore mitosis was significantly more efficient than the conventionally used techniques.

Key words: Wheat – Anther culture – Colchicine – Chromosome duplication – Fertility of regenerants

Introduction

In genetic and plant breeding programs, a large number of haploid plants that can easily and efficiently be transposed into genetically stable dihaploids is required. *Triticum aestivum* L. anther culture can provide a number of haploid plants (De Buyser and Henry 1980; He and Ouyang 1984; Liang et al. 1987; Barnabás et al. 1989), but an intensive effort should be made to develop effective methods to produce true homozygotes, either by doubling the haploid initial or by doubling the chromosome complement of the verified haploid plants. Efficient methods must have high doubling rates, low risk of damage, and must be easily applicable in practice.

Colchicine is the most frequently used chromosome doubling agent. It stops mitosis at metaphase by inactivation of the spindle mechanism (Levan 1938; Jensen 1974). In general, the treatments are carried out on the whole plants by soaking the crown for several hours in colchicine solution (Henry and De Buyser 1980). The colchicine treatment to the plants is rather complicated and can cause high mortality due to the toxic effect of the agent. The developmental stage of the exposed plants may also have an important role in the effectiveness of the treatment. Zhuang and Jia (1980) increased the diploid plant percent by soaking the haploid callus for 72 h in colchicine solution (0.01–0.04%) before transfering them to regeneration medium.

Generally, to obtain a nonchimeric doubled haploid one would have to induce doubling at the single-cell stage. There have been several attempts to treat the anthers of different plant species (tobacco, rice, rubber tree, poplar, etc.) with colchicine added to the culture media (Nitsch 1977; Ying 1986; Hu and Liang 1979; Wu et al. 1980). In this method, the percent of diploid pollen plants was raised but, in most cases, the callus induction frequency as well as the plant differentiation frequency declined. Data on wheat were not found at this time.

The aim of the present work was to study the direct effect of colchicine on the androgenic development of the microspores in *Triticum aestivum* L. anthers and to compare the effectiveness of the different doubling procedures on the fertility of the microspore-derived wheat plants.

Materials and methods

Anthers of *Triticum aestivum* L. (cv 'Ciano') containing microspores at mid- or late-uninuclear stage were inoculated on a P2 medium (Chuang et al. 1978) under aseptic conditions. Four concentrations (0.00, 0.01, 0.02, 0.04%) of colchicine were added to the induction medium. The anthers were incubated for 3 days on this medium at 29 °C and then transferred to a colchicine-free P2 medium for further culturing. The cultures were then incubated

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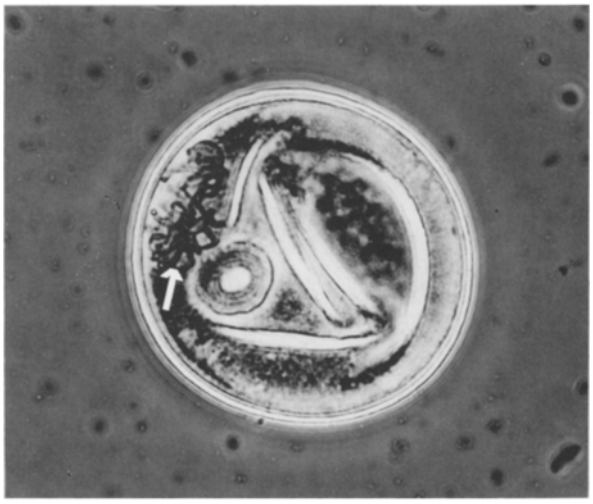

Fig. 1. Astral arrangement of the chromosomes (indicated by the *arrow*) in dividing microspore cultured on the medium containing 0.02% colchicine

for 40 days in the dark. A minimum of 500 anthers was used in each treatment. After 5 weeks, callus and pollen embryo formation was determined. The induction frequency of haploid structures was calculated on the basis of responding anthers. Plants were regenerated from pollen calli or embryoids on a 190-2 medium (He and Ouyang 1984) at a temperature of 26 °C with 16-h illumination. The plant regeneration percentage was counted on the basis of pollen embryos.

After vernalization (45 days at 2 °C), the regenerants (R_0) were grown in growth chambers (Conviron GB type) at a constant temperature of 16 °C with a 16-h light/8-h dark photoperiod (290 μmol m^{-2} s^{-1} photon flux density). One part of the plants regenerated from the untreated anthers was exposed to the conventional colchicine treatment used in our laboratory; that is, in the five-leaf stage of development, the root system and the shoot apex of the plantlets were immersed into 0.04% colchicine solution for 12 h at 15 °C. Other plants were grown to maturation without any treatment. After harvesting, the fertility of the plants was checked and compared. The seeds of three plants from each treatment were then grown in a field experiment to study the next (R_1) generation for its fertility and some related characters.

Results

Anther response and plant regeneration

Colchicine applied to the induction medium caused 'c-mitosis' in certain microspores (Fig. 1). The astral arrangement of the mitotic chromosomes is typical of colchicine action. As the data of Table 1 show, there was a slight reduction in the anther response due to the increased colchicine concentration in the medium, but the differences were not significant. The induction of haploid structures in the responding anthers was similar to the control in each treatment. The anthers cultured in the presence of colchicine produced a significantly higher percent of pollen embryos than that of calli. The fre-

Table 1. Effect of different colchicine concentrations on the androgenic ability of the cultured anthers

Concentration (%)	No. of anthers cultured	Anther response %	Induction of haploid structures %	% of pollen embryos
0.00	528	20.3	202.0	20.8
0.01	552	15.9[a]	210.0[a]	82.2***
0.02	501	12.4[a]	189.0[a]	77.8***
0.04	528	11.7[a]	237.0[a]	86.4***

[a] Not significant from 0.00% concentrations at $P=0.05$
*** Significantly different from 0.00% concentration at $P=0.001$

Table 2. Plant regeneration from pollen embryos induced in the presence of colchicine

Concentration (%)	No. of embryos cultured	% of plant regeneration	% of albino plantlets
0.00	110	81.6	14.1
0.01	124	80.7[a]	6.5[a]
0.02	74	79.4[a]	4.1*
0.04	103	84.4[a]	1.9**

[a] Not significant from 0.00% concentration at $P=0.05$
*, ** Significantly different from the 0.00% concentration at the 0.05 and 0.01 probability levels, respectively

Table 3. Effect of different chromosome doubling techniques on the fertility of the regenerants (R_0)

Treatment		No. of plants examined	% of fertile plants	Mean no. of seeds/plant
0.00%	colchicine[a]	39	46.2	55
0.01%	colchicine[a]	48	52.1*	42
0.02%	colchicine[a]	35	68.8*,**	58*,**
0.04%	colchicine[a]	58	67.2*,**	64*,**
	conventional[b]	24	20.8*	31*

[a] Added to the induction medium
[b] Young plantlets treated with 0.04% of colchicine
* Significantly different from 0.00% at $P=0.05$
** Significantly different from the conventional at $P=0.05$

Table 4. Fertility as measured by seed set percent of the field-grown R_1 progeny

Origin of the plants	Seed set %[a]
Cultivar control	70.2
Spontaneously doubled	63.9
Conventionally doubled	50.3
0.01% colchicine doubled	66.6
0.02% colchicine doubled	59.5
0.04% colchicine doubled	63.6

[a] LSD values were 10.5, 14.4, and 19.2 at the 0.05, 0.01, and 0.001 probability levels, respectively

quency of embryo formation was considerably lower in the case of untreated anthers.

The plant regeneration ability of the cultured pollen embryos was not influenced by colchicine (Table 2). However, the ratio of albino plantlets was significantly reduced by colchicine, especially at a concentration of 0.04%.

Fertility of the regenerated plants (R_0) doubled by various methods

The results are summarized in Table 3. The data indicate the relatively high spontaneous doubling was present in the genotype. Almost 50% of the regenerants produced seeds without any treatment, and the mean seed number of the fertile plants was also relatively high. In the case of the conventional treatment, both the fertility and the seed production were significantly lower then in the other treatments examined. When colchicine (particularly in 0.02 and 0.04% concentrations) was added to the induction medium, the chromosome doubling resulted in a significantly higher number of fertile plants and increased seed production.

Fertility of the next (R_1) progeny

The next (R_1) generation that was grown in the field showed the same tendency concerning the seed set of plants (Table 4). The original cultivar gave the highest seed set for fertility. Fertility of the conventionally treated offspring was improved, possibly due to the selection in the parents, but it still was considerably lower than that of the others. Seed set in the progeny of the spontaneously doubled plants and of the colchicine-treated microspores was quite similar.

Discussion

Successful chromosome doubling of microspore-derived haploids is necessary for propagating homozygous plants. To ensure homozygosity, genome doubling should preferably occur before the first microspore mitosis of the microspores in cultured anthers.

In the present experiment, the chromosome doubling due to the direct effect of colchicine on the young microspores was shown to be effective. The induction frequency of microspore-derived haploid structures was not greatly influenced by colchicine, which is important in this technique is to be used in practical wheat breeding.

The increased number of pollen embryos of the medium containing colchicine supports the observations of Zaki and Dickinson (1990) that the frequency of symmetrically dividing microspores, which might be the origin of the haploid embryos, increased when 25 mg/l of colchicine was added to *Brassica napus* L. microspore cultures for 12 h. Other experiments have also indicated that embryogenic development is regularly preceded by symmetrical division of the microspore (Fan et al. 1988; Szakács and Barnabás 1988). These results suggest that alteration of division symmetry by suppression of the formation of microtubular architecture in the premitotic microspores is sufficient to switch the developmental pathway to embryo formation. Since the regulation of the sporophytic and gametophytic pathway in anther or microspore cultures is not clear, studies on the action of an antimicrotubule agent might be important for both fundamental and applied approaches. We can point out that the significant decrease in the number of albino plantlets might indicate the probable selective effect of the colchicine for the microspores which were capable only of albino plant production.

Colchicine applied during the induction phase resulted in significantly higher fertility of the regenerated (R_0) plants. The seed production of the plants was improved especially at the 0.02 and 0.04% colchicine concentrations. In the genotype studied, the spontaneous doubling rate was high. With the lack of critical cytological observations, it is not known at which stage of the in vitro culturing the chromosome doubling occurred. It is also not clear whether spontaneously doubled genomes differ from chemically doubled genomes in the degree of genetic stability.

The surprisingly low fertility of the conventionally treated plants could be explained by differences among cells for chromosome doubling in the treated organized tissue. The success of colchicine treatment applied to the plant can depend on many internal and external factors (physiological conditions and developmental stage of the plants, environmental conditions, etc.), which put some limitations on the reliable application of the technique.

The progeny (R_1) of three R_0 regenerants grown in the field showed the same tendency in their fertility as the previous generation.

The action of colchicine on the cultured microspores seemed to be efficient, and the plants (R_0 and R_1) originating from the doubled microspores had the great advantage of being fertile. However, the critical analysis of the applied treatments can only be done after examining the critical analyses of the R_2 generation, which is in progress.

As the induction of variation is important in plant breeding, the availability of variation and the aim of the breeding program will determine which method or combination of methods should be used for cultivar development.

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