Theoretical and Applied Genetics 46, 407-410 (1975)

In vitro Culture of Wheat. III. Anther Culture of the A Genome Aneuploids in Common Wheat *

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Summary. Callus induction was examined using the anthers of *Triticum aestivum* cv. Chinese Spring and its aneuploids for the A genome chromosomes (ditelo-1A^L, -2A^S, -3A$^\alpha$, -4A$^\alpha$, -5A^L, -6A$^\alpha$, -7A^S, nulli-1A, -2A, -4A and mono-4A) to determine the genetic role of individual chromosomes or chromosome arms in callus formation. An attempt was also made to establish the best culture conditions for callus formation from the anthers of the same wheat.

Results showed that: (1) Callus was mostly induced from the anther filament. The frequency varied with the strain: ditelo-4A$^\alpha$ showed the highest frequency (41.3 %), followed by nulli-4A (16.9 %), in contrast to low frequencies for the normal strain and the other aneuploid strains. Undoubtedly, some genetic factor(s) for inhibiting callus induction is located on the β arm of chromosome 4A. (2) 2,4-D was needed to induce callus, but IAA and kinetin had no effect. 3 % sucrose was more favourable than 6 % for callus formation. Anthers cultured at the middle-uninucleate stage produced calluses more easily than those cultured at the other uninucleate stages (early or late). (3) A callus originating from the pollen of nulli-2A was obtained. From this, many albinotic plantlets were produced on a medium containing no 2,4-D.

Introduction

Haploids of various plants have been obtained by anther culture (e.g. Guha and Maheshwari 1964). Recently, Ouyang et al. (1973) succeeded in inducting haploids of *Triticum aestivum* by this method. We too have carried out the anther culture of wheat, but found callus formation from anthers to be very diffucult in this crop (Shimada 1972). Supposing that there are some genetic factors which control callus formation (Tabata and Motoyoshi 1965), certain strains lacking the chromosome(s) or chromosome segment(s) which carries the genetic factor(s) will differ from the normal strain in the feasibility of callus induction. In the present investigation, we tested callus formation from the anthers of different kinds of common wheat aneuploids for the A genome chromosomes, to determine the genetic role of these chromosomes or chromosome arms in callus formation. We also tried to establish proper culture conditions for callus formation from the anthers of the same wheat.

Materials and Methods

Materials used were a common wheat *Triticum aestivum* cv. Chinese Spring (2n = 42, genome constitution AABBDD) and its aneuploid derivatives, ditelo-1A^L, -2A^S, -3A$^\alpha$, -4A$^\alpha$, -5A^L, -6A$^\alpha$, -7A^S, nulli-1A, -2A, -4A and mono-4A. The aneuploid stocks were kindly supplied by Dr. E.R. Sears of the University of Missouri, and have been maintained in our laboratory with a check of their karyotypes every generation.

Anthers at the uninucleate stage were used for inoculation. They were further divided, in part of this experiment, into three classes, the early, middle and late uninucleate stages as defined by Ouyang et al. (1973). The stages of pollen development were identified by the aceto-carmine smear method.

The inoculation was performed as follows: young spikes were immersed in 5 % calcium hypochlorite for 20 - 30 minutes, then washed five times with sterilized water; anthers were removed from the florets and inoculated on a culture medium under aseptic conditions. The basal medium of Blaydes (1966) or RM-64 (Linsmaier and Skoog 1965) was supplemented with 2,4-D, IAA and/or kinetin as indicated in Table 2. Inoculated test tubes were kept at 25°C under fluorescent light. To induce shoot formation, the callus formed from the pollen was transferred to a medium not containing 2,4-D.

The number of chromosomes in the callus cell was determined by pretreating calluses with ice water (0°C) for 24 hours, fixing with 1:3 acetic alcohol and hydrolyzing with 1 N-HOl; they were then stained with Feulgen's leuco-basic fuchsin and squashed (rf. Shimada et al. 1969).

Results

1. Callus formation

Difference among strains: Anthers at the uninucleate stage of ditelosomics, monosomics and nullisomics were cul-

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tured on RM-64 or Blaydes basal medium containing 2 mg/l 2,4-D. The results are summarized in Table 1. Since no difference was found between the two basal media, the results were combined. The frequency of callus formation differed remarkably among the strains used. Of all the strains tested, ditelo-4A$^{\alpha}$ showed the highest frequency (41.3 %), followed by nulli-4A (16.9 %), both of which totally lacked the β arm of chromosome 4A. Most strains showed frequencies lower than 10 % . All calluses, except a single one from nulli-2A, were produced from the anther filament, and their cells had the same chromosome numbers as the original stocks (2n = 40 - 42).

Table 1. Callus induction from the anthers of ditelo-, mono- and nullisomics of Chinese Spring for the A genome chromosomes

Strain	No. anthers cultured	Callus formation No.	%	95 % confidence interval (%)
Normal	356	9	2.5	1 - 6
Ditelo-1A^{L}	128	5	3.9	1 - 9
-2A^{S}	12	0	0	0 - 24
-3A$^{\alpha}$	98	11	11.2	5 - 19
-4A$^{\alpha}$	184	76	41.3	33 - 49
-5A^{L}	200	2	1.0	0 - 4
-6A$^{\alpha}$	94	11	11.7	5 - 19
-7A^{S}	64	1	1.6	0 - 7
Mono-4A	60	4	6.7	2 - 12
Nulli-1A	44	2	4.5	0 - 16
-2A	12	1	8.3	0 - 36
-4A	89	15	16.9	11 - 28
Total	1,341	137	10.2	-

Effect of growth factors: Effects of kinetin (1 - 2 mg/l and IAA (2 mg/l) on callus induction from the anthers of some ditelosomics were tested, using Blaydes basal medium containing 2 mg/l 2,4-D. The results are shown in Table 2. In no case did a supplement of IAA and/or kinetin to the medium induce calluses. Undoubtedly, kinetin and IAA at these concentrations inhibit callus formation from wheat anthers. It is known that casein hydrolysate, coconut milk and yeast extract have no effect on callus formation (Shimada 1972).

Table 2. Effects of 2,4-D, IAA and kinetin on callus induction from the anthers of Chinese Spring ditelosomics for the A genome chromosomes

Strain	Medium*	No. anthers cultured	Callus formation No.	%
Ditelo-1A^{L}	1,2,3,4	140	0	0
-2A^{S}	1,2,3,4	67	0	0
-3A$^{\alpha}$	1	27	1	3.7
	2,3,4	75	0	0
-4A$^{\alpha}$	1	27	17	62.9
	2,3,4	87	0	0
-6A$^{\alpha}$	1,2,3,4	63	0	0
-7A^{S}	1,2,3,4	70	0	0
Normal	1	308	7	2.2
	2,3,4	220	0	0

* 1: Blaydes basal medium + 2 mg/l 2,4-D
2: (1) + 1 mg/l kinetin
3: (1) + 2 mg/l kinetin
4: (1) + 2 mg/l kinetin + 2 mg/l IAA

Effect of the sucrose concentration: The effect of the sucrose concentrations (3 and 6 %) on callus induction was tested, using the RM-64 basal medium supplemented with 2 mg/l 2,4-D. Results are shown in Table 3. The

Table 3. Effects of two concentrations of sucrose on callus induction from the anthers of ditelosomics and nullisomics

Strain	Sucrose concentration					
	3 %			6 %		
	No. anthers cultured	No. calluses formed	%	No. anthers cultured	No. calluses formed	%
Nulli-1A	24	2	8.3	20	0	0
-2A	6	1	16.7	6	0	0
-4A	45	11	24.4	44	4	9.0
Ditelo-1A^{L}	20	2	10.0	36	2	5.6
-3A$^{\alpha}$	16	1	6.3	16	0	0
-4A$^{\alpha}$	56	19	33.9	56	14	25.0
-7A^{S}	4	0	0	9	2	22.2
Total	171	36	21.1	187	22	11.8

over-all frequency of callus formation was 21.1 and 11.8% for 3 and 6% sucrose, respectively. The difference was significant. All the calluses produced were also derived from the anther filament, except for one from nulli-2A, and were obtained on the medium containing 3% sucrose. Apparently, 3% sucrose gives better results for callus induction from anther filaments by 2,4-D.

Difference due to the stages: To find out the proper stage of inoculation for callus induction, anthers of ditelo-4A$^{\alpha}$ at the early, middle and late-uninucleate stages were cultured on RM-64 basal medium containing 2 mg/l 2,4-D. Results are shown in Table 4. Anthers cultured at the middle-uninucleate stage showed a higher frequency (41.2%) of callus induction from anther filament than did those cultured at the other two stages (29.2 - 25.0%), though the difference was not statistically significant because of small sample size. No callus was derived from the pollen grain.

Table 4. Callus formation from the anthers of ditelo-4A$^{\alpha}$, which were cultured at three different uninucleate stages

Stage	No. anthers cultured	Callus formation No.	%
Early-uninucleate	24	7	29.2
Middle- "	34	14	41.2
Late- "	8	2	25.0

2. Haploid plants

Almost all the calluses obtained in this experiment were derived from the anther filament, but one callus was obtained from the pollen of nulli-2A (2n = 40), as mentioned above. This callus was relatively hard and yellowish in appearance, in contrast to the soft, white calluses of anther filament origin. The chromosome number of the cells varied from 19 to 21 with the mode at 21 (Fig.1a). To restore plants from this callus, part of it was transplanted to a medium containing no 2,4-D. After one month many shoots and roots were formed from the callus. Unfortunately, all the plantlets were albinotic (Fig.1b). These albinotic plants were considered to be haploid, because their root-tip cells had 21 chromosomes. They grew to the three or four leaf stage (Fig.1b), but withered and died about six months after callus inoculation.

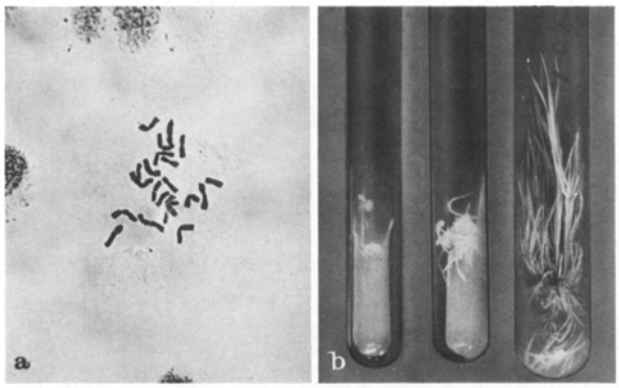

Fig.1. (a) Mitotic metaphase of a callus cell (21 chromosomes), originating from an anther of nulli-2A (2n=40). The chromosome number of the callus cells of this stock was 21 at the mode, with a range of 19 to 21. (b) Left: Callus derived from an anther (possibly from pollen) of nulli-2A; Middle: Roots and albinotic shoots reorganized from a callus of anther origin on medium containing no 2,4-D; Right: Albinotic plantlets after four months of culture

Discussion

Ouyang et al. (1973) obtained calluses of pollen origin from various F_1 hybrids of *Triticum aestivum* with a frequency as high as 3.1% in "Kochum 5 × Hsiaoyen 759" F_1, but the number and type of the calluses varied considerably among the different materials. Using a cultivar, 'Chinese Spring', of the same species we obtained only one pollen callus from 1,341 anthers cultured, though the medium and pollen stages we used were similar to theirs. Apparently, different strains (or cultivars) of common wheat show quite different responses to the same culture conditions.

The frequency of callus induction from the anther filament was significantly higher in ditelo-4A$^{\alpha}$ than in the other ditelosomics. A comparatively high frequency of callus induction was also noticed in nulli-4A. This indicates that some genetic factors inhibiting callus formation from the anther filament of common wheat are located on the β arm of chromosome 4A. The fact that the frequency of nulli-4A was lower than that of ditelo-4A$^{\alpha}$ seems to suggest that some factors promoting callus induction are located on the α arm of chromosome 4A. This could be further tested by using ditelo-4A$^{\beta}$, which was not available for the present investigation. It has been shown for some plants that the ease of callus formation varies among the different strains and varieties. For example, Tabata and Motoyoshi (1965) suggested that the waxy strain of maize possesses a

genetic factor for inhibiting callus formation from the endosperm explant. The present study has provided the first evidence for the chromosomal location of a genetic factor controlling callus induction.

A high concentration of sucrose is known to have a favourable effect on callusifying the pollen of wheat (Ouyang et al. 1973) and barley (Clapham 1973). In this experiment, however, this effect was not observed: only one pollen callus was induced on the medium containing an ordinary concentration of sucrose (3%). As to the callus of anther filament origin, the most favourable condition for callus induction was to culture anthers at the middle-uninucleate stage on a medium containing 2 mg/l 2,4-D and 3% sucrose.

The modal chromosome number of the callus cells derived from the pollen of nulli-2A was 21, instead of 20, a haploid number of nullisomics. Since the nulli-2A shows considerable asynapsis at meiosis (Sears 1954), it should produce pollen grains with 21 chromosomes at a certain frequency. The present callus is assumed to have been derived from such pollen.

It is very interesting and surprising to note that plants restored from callus of pollen origin in various cereals, such as *Hordeum* (Clapham 1973), triticale (Ono and Larter 1973), *Aegilops* (Kimata and Sakamoto 1972) and *Triticum* (Ouyang et al. 1973 and the present investigation), mostly became albinotic. Clapham (1973) suggested that the frequency of chlorophyll-deficient plants could be altered by changing the composition of the culture medium. The present experiment did not provide any positive information on this proposal, nor were there any clues to a possible explanation for their origin. Further studies are needed.

Acknowledgements

We are greatly indebted to Dr. K. Tsunewaki for his critical reading of this manuscript.

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Received March 8, 1975
Communicated by H. Kihara

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