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The effect of specific chromosome and cytoplasm substitutions on the tissue culture response of wheat (*Triticum aestivum*) callus

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Summary. Calli were initiated from immature embryos of four lines of hexaploid wheat (*Triticum aestivum* L. em. Thell), the euplasmic nuclear donor 'Chinese Spring', 'Chinese Spring' in which both 4B chromosomes were substituted by those of the variety 'Cappelle-Desprez' and two alloplasmic lines in which these nuclei were substituted into the cytoplasm of *Aegilops ovata*. The calli were found to differ in their initial growth rates and their ability to organise shoot primordia and regenerate shoots. The 'Cappelle' 4B chromosomes had a very significant effect on all these characters. The potential for modelling genotypes for improved tissue culture characteristics is discussed.

Key words: Wheat – Callus – Regeneration – Chromosomes – Cytoplasm

Introduction

Bingham et al. (1975) provided the first evidence that regeneration of shoots from callus cultures was genetically controlled and that this control could be manipulated through conventional breeding techniques. Starting from alfalfa lines which were poor regenerators, they used recurrent selection to establish lines which differed significantly from one another with respect to their regeneration capacity. Over three generations they were able to increase regeneration activity from 12% to 67%.

Several authors have reported genotypic effects on the growth of wheat in culture. The frequency of callus initiation from anthers and the growth rate of calli from roots and shoot nodes both show significant variation between aneuploid lines of 'Chinese Spring' wheat (Shimada and Makino 1975; Barone-celli et al. 1978). It was Shimada (1978) who first reported variation in the frequency of shoot regeneration from calli of different wheat cultivars. Recently there have been reports (Sears and Deckard 1982; Lazar et al. 1983; Maddock et al. 1983) that significant differences occur in the tissue culture responses of calli initiated from immature embryos of breeders' lines and commercial varieties of spring and winter wheats. The use of tissue culture as a tool in the genetic improvement of the cereals has been seriously restricted by the recalcitrance of most of the Graminaceous species in protoplast, callus, and anther culture. Previously much effort has been expended in the search for culture conditions (Lazar et al. 1983; Nabors et al. 1983) and genotypes (Sears and Deckard 1982; Lazar et al. 1983) which might provide the key to efficient handling of these species in culture. The observed genotypic differences suggest that wheat tissue culture performance might be improved by the directed genetic improvement of lines for use in tissue culture programmes. Such an approach is greatly facilitated by the existence of a large number of chromosome substitution and aneuploid lines in wheat.

In this paper we report the effect of specific chromosome and cytoplasm substitutions on the tissue culture responses of calli of four lines of 'Chinese Spring' wheat.

Materials and methods

Plant material

These experiments used euplasmic lines of the nuclear donor 'Chinese Spring' (CS), a line in which the 4B chromosomes of CS are substituted by the 4B chromosomes of 'Cappelle Desprez', CS (Cap 4B), and two lines in which these nuclear genomes are substituted into the cytoplasm of *Aegilops ovata* ((*ovata*)-CS and (*ovata*)-CS(Cap 4B), respectively).

The lines were produced and are maintained at the Plant Breeding Institute, Cambridge.

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Plants were grown as previously described (Mathias et al. 1985). Individual ears were bagged at ear emergence and allowed to self-pollinate, the date of anthesis of each ear was noted.

Tissue culture

The callus cultures were initiated and maintained according to Mathias et al. (1985). The basal medium was that described by Sears and Deckard (1982). The initiation, maintenance and regeneration media differed only in the concentration of 2,4-D they contained (1.0, 0.5 and 0.1 mg/l, respectively).

The callus growth on initiation medium was recorded as total callus area per petri dish. On initiation and maintenance medium the number of calli with green spots was recorded. On maintenance and regeneration medium the number of calli which produced shoots was recorded.

Results

The initiation, maintenance and regeneration media differed only in the amount of 2,4-D they contained. Therefore, the growth responses of calli on particular media must essentially be responses to a particular concentration, or change in concentration, of 2,4-D and differential responses must reflect specific interaction of the cytoplasm and/or genotype with the growth regulator in the medium.

In all lines callus growth from the embryo/scutellar explants became visible after five to seven days on initiation medium, there were no obvious differences in the time of appearance of callus. The substitution of the *Ae. ovata* cytoplasm or the Cap4B chromosome had no effect on callus induction as there was no variation between lines and every one of the excised embryos cultured under the described conditions produced calli. All calli were of similar appearance, typically pale yellow-white in colour with a loose friable texture.

The growth rate of calli on initiation medium (measured as callus area on a per petri dish basis) was significantly increased by the substituted Cap4B chromosome and significantly reduced by the *Ae. ovata* cytoplasm (Table 1).

All lines showed the same general morphogenic responses in culture. Production of shoots and shoot primordia was inversely related to 2,4-D concentration, a pattern that agrees with previous reports (Shimada 1978; Sears and Deckard 1982; Maddock et al. 1983; Mathias et al. 1985).

On all media some calli of each line developed green shoot primordia. On initiation medium primordia formation was affected by both the *Ae. ovata* cytoplasm and the Cap4B chromosome. The development of shoot primordia was significantly depressed in the alloplasmic lines, while the chromosome substitution lines produced significantly more primordia than the euploid lines (Table 2).

In all lines transfer to maintenance medium resulted in a very significant increase in the number of calli with green spots. On maintenance medium the Cap4B lines continued to produce more primordia than the euploids but the cytoplasm no longer affected primordia formation.

After one month on maintenance medium some calli of all lines had produced shoots. The Cap4B chromosome very significantly increased the proportion of calli that regenerated shoots (Table 3).

Table 1. Callus growth rate on initiation medium, (total area in mm^2 of callus/petri dish – 10 calli/dish)

Lines	Means	Reps
CS	140.6	11 a
CS(Cap4B)	167.2	11 ab
(*ovata*)-CS	131.1	12 bc
(*ovata*)-CS(Cap4B)	145.0	10 bc

Lines followed by the same letter are significantly different from the first line with that letter (analysis of variance)

Chromosome and cytoplasmic effects on initial callus growth on initiation medium		
aestivum v *ovata* cytoplasm	df. 1	MS. 2,662.7**
CS4B v Cap4B chromosome	df. 1	MS. 4,850.2***
Interaction	df. 1	MS. 440.6 ns
Error	df. 40	MS. 224.4

Table 2. Percentage morphogenic calli on initiation medium and maintenance medium

Line	Initiation	*n*	Maintenance	*n*
CS	44.5	110	68.3	104 ***
CS(CAP4B)	56.4	110	92.0	102 ***
(*ovata*)-CS	23.3	120	71.0	119 ***
(*ovata*)-CS(Cap4B)	47.0	100	82.5	100 ***

Calli of all lines had significantly more green spots on maintenance medium than on initiation medium (Chi^2 analysis)

Chromosome and cytoplasmic effects on callus morphogenesis on initiation medium		
Chi^2 heterogeneity test	df. 3	27.74***
aestivum v *ovata* cytoplasm	df. 1	12.07 ***
CS4B v Cap4B chromosomes	df. 1	15.27 ***
Interaction	df. 1	0.39

Chromosome and cytoplasmic effects on callus morphogenesis on maintenance medium		
Chi^2 heterogeneity test	df. 3	22.33 ***
aestivum v *ovata* cytoplasm	df. 1	0.63 ns
CS4B v Cap4B chromosomes	df. 1	18.96 ***
Interaction	df. 1	2.73

Table 3. Percentage regenerable calli on maintenance and regeneration medium

Line	Maintenance	*n*	Regeneration	*n*
CS	25.0	104	48.4	93 ***
CS(Cap4B)	47.0	102	58.2	64
(*ovata*)-CS	26.9	119	38.5	117
(*ovata*)-CS(Cap4B)	52.5	100	57.7	100

Calli of CS regenerated significantly more shoots on regeneration medium than on maintenance medium (Chi² analysis)

Chromosome and cytoplasmic effects on callus regeneration on maintenance medium		
Chi² heterogeneity test	df. 3	27.96 ***
aestivum v *ovata* cytoplasm	df. 1	0.69 ns
CS4B v Cap4B chromosomes	df. 1	27.18 ***
Interaction	df. 1	0.09

Chromosome and cytoplasmic effects on callus regeneration on regeneration medium		
Chi² heterogeneity test	df. 3	10.83 *
aestivum v *ovata* cytoplasm	df. 1	0.73 ns
CS4B v Cap4B chromsomes	df. 1	8.78 **
Interaction	df. 1	1.31

After one month on regeneration medium an increased proportion of the calli of all lines produced shoots. This increase was significant only in CS. The Cap4B chromosome had a significant effect on shoot regeneration activity on regeneration medium (Table 3). The cytoplasm had no effect on shoot regeneration activity on maintenance or regeneration medium, as reported by Mathias et al. (1985).

There was no interaction between the *Ae. ovata* cytoplasm and the Cap4B chromosome for any of the characters studied.

Discussion

The cytoplasm of *Ae. ovata* and the 4B chromosome of 'Cappelle-Desprez' affect the response of callus cells to 2,4-D at concentrations routinely used in initiating, maintaining and regenerating wheat calli. The *Ae. ovata* cytoplasm seems to increase the sensitivity of cell growth and primordia organisation to the inhibitory effects of 2,4-D. The substitution of the Cap4B chromosome, either through the introduction or removal of a gene or genes, results in an increase in cell proliferation and shoot regeneration which probably reflects reduced sensitivity to 2,4-D.

The 2,4-D concentrations in the initiation and maintenance media (1.0–0.5 mg/l, respectively) were originally chosen to inhibit shoot regeneration and promote callus development (Sears and Deckard 1982). That morphogenic activity is suppressed in calli on high levels of 2,4-D is demonstrated by the increased production of shoot primordia and shoots that occurs in all lines on transfer to media with less 2,4-D.

On initiation and maintenance media the cytoplasmic lines showed reduced cell proliferation and organisation of primordia. This perhaps reflects the 'hybrid' nature of the cells, partial incompatibility of the non-adapted CS nucleus and *Ae. ovata* cytoplasm would reduce cell fitness, which might render calli both more sensitive to exogenous 2,4-D and more readily inhibited on exposure to supra-optimal 2,4-D concentrations. On a low concentration of 2,4-D (0.1 mg/l) the cytoplasm had no effect on tissue response, this level may be sub-optimal for cell and tissue differentiation.

Cell proliferation, primordia formation and shoot regeneration in calli of the Cap4B chromosome substitution lines occurred, and was more active, at higher 2,4-D levels than in the euploid lines. This improved performance may result from the introduction of a growth stimulating gene(s) on the Cap4B chromosome or the loss of an inhibitory gene(s) on the CS 4B chromosome. Baroncelli et al. (1978) has reported that root calli of a CS line ditelocentric for the 4B long arm grew more slowly than calli of the euploid. Unfortunately stem node calli of the same line did not show depressed growth. This contradictory evidence at best suggests that there may be a gene on the 4B chromosome of CS which promotes culture growth. If this is so, then our results suggests that there may be a stronger promoter on Cap4B.

The nature of this promoter is unknown. Interestingly the homoeologous group 4 chromosomes carry some of the most potent 'whole plant phenotype' genes that have been identified in wheat. The 'grass clump dwarfness' allele D3 (Hermsen 1963) has been mapped to 4B. The mode of action of this gene is not known but as it causes a squat habit with many tillers it must operate through the disturbance of normal hormone metabolism and shoot meristem formation. The homoeologous chromosomes 4A and 4D carry the reduced height/gibberellic acid insensitivity alleles, *Rht1* and *3* and *Rht2* respectively (Gale 1979). These genes also disturb the hormone metabolism of the plant creating a semi-dwarf phenotype. It is possible that an allele at the 'grass clump dwarfness' locus D3 or the 4B equivalent of the *Rht* loci of chromosomes 4A and 4D accounts for improved tissue culture performance of Cap4B lines through an effect on cellular hormone metabolism.

This is the first report of the involvement of a specific chromosome in the control of morphogenesis and shoot regeneration in tissue culture. Further experiments are in progress to establish which arm of the chromosome carries the gene(s) responsible for the effects we have observed and whether the effects result from the addition of a promoter or the removal of an inhibitor.

Understanding the genetic basis of culture behaviour is essential to any long term project to develop lines which are especially suited to particular tissue culture systems. It may be that the only means of increasing the efficiency of anther and protoplast cul-

ture in recalcitrant species such as wheat, is to select genetic stocks which perform well in these systems. We are at present investigating the culture characteristics of several varieties which are parent varieties for chromosome substitution series, these series will enable us to screen all of the chromosomes of wheat for activity in tissue culture.

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