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Aneuploid and alloplasmic lines as tools for the study of nuclear and cytoplasmic control of culture ability and regeneration of scutellar calli from common wheat

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Summary. Twenty four B genome aneuploid lines (ditelosomics, nullisomic-tetrasomics and tetrasomics) of *Triticum aestivum* cv 'Chinese Spring' were used in an analysis of the culture ability and regeneration capability of scutellar calli. Several correlations were found between the presence or absence of specific chromosomes and chromosomal arms of the B genome of common wheat and the growth and differentiation capabilities of these calli. The rate of callus growth decreased only when the long arm of chromosome 6B was not present. The absence of chromosomes 3B and 7B did not result in an apparent change in morphogenetic capability, while the absence of other B genome chromosomes was significantly correlated to changes in the frequency of calli that regenerated plants. The presence of the short arm of chromosome 1B was negatively correlated with regeneration, whereas its long arm is probably required to counteract this effect and to maintain the normal ratio of regeneration. The presence of the chromosomal arm 2BS seemed to be essential for differentiation to shoots. In the absence of the short arms of chromosomes 4B and 5B, the rate of regeneration was slightly reduced. In the absence of the long arm of chromosome 6B there was a marked reduction of the ability of scutellar calli to regenerate plants. The use of additional aneuploid lines belonging to homoeologous group 6 revealed that only calli derived from lines having chromosome 6D in their complement regenerated plants similarly to the euploid control. Culture ability and regeneration capability were also analysed with alloplasmic lines of *T. aestivum* cv 'Chris'. The lines were derived from five species, representing plasma-types of different phylogenetic distances from plasma-type B of *T. aestivum.* The results showed that when the endogenous cytoplasm (B-type) was exchanged with *T. timopheevii* cytoplasm (G-type) there was a significant increase in the regeneration of shoots from the scutellar calli.

Key words: *Triticum* – *Aegilops* – Scutellar callus – Aneuploids – Alloplasmic lines

Introduction

Tissue-culture ability is a broad term used to describe a complex character of plants rendering them amenable to in vitro culture. This 'trait' is probably comprised of several control mechanisms which regulate successive steps of the culture cycle. The initiation of callus, callus growth, differentiation to root or shoot primordia and finally the regeneration of plants were repeatedly suggested to be under genetic control, but the genetic basis of this control requires further clarification.

The genetic component of culture ability was demonstrated by selection of callus with high regeneration capability from poorly regenerating callus (Bingham 1975) and by the results of diallel crosses between genotypes having consistent differences in performance in culture (e.g. Keyes et al. 1980; Frankenberger et al. 1981; in callus and anther culture of cereals: Bullock et al. 1982; Lazar et al. 1984; Beckert and Qing 1984; Charmet and Bernard 1984; Tomes and Smith 1985; Miah et al. 1985).

Only a few specific genetic influences on culture ability of plant tissues were reported, such as the introduction of enhanced morphogenetic capability of specific species to interspecific and intergeneric F_1 hybrids (Orton 1979; Nakamura et al. 1981, respectively) or the contribution of specific genomes to the in vitro performance of polyploid species (Cheng and Smith 1973; Ogura and Tsuji 1977).

In common wheat, consistent differences in culture ability of immature-embryo-derived scutellar callus have been demonstrated among species and cultivars (e.g. Shimada 1978; Gosch-Wackerle et al. 1979; Sears and Deckard 1982; Maddock et al. 1983). Specific chromosomes which were highly correlated with culture ability were reported mainly in common wheat (Shimada and Makino 1975; Baroncelli et al. 1978; Henry and de Buyser 1985; Mathias and Fukui 1986).

Differences detected between the in vitro performance of explants obtained from reciprocal crosses led to the suggestion that the plasmone (i.e. the 'cytoplasm', without specific reference to either the chloroplast or the mitochondrial genomes) may also influence the culture ability of plant tissues (Mitchell et al. 1980; Rauqin 1982; Foroughi-Wehr et al. 1982; Lazar et al. 1984; Charmet and Bernard 1984). In wheat cell-culture, few studies using alloplasmic lines are available which also indicate the possible effect of the organelles on tissue culture ability (Picard et al. 1978; Kinoshita and Mikami 1984; Mathias and Fukui 1986; Mathias et al. 1986).

The examination of the culture ability of scutellar calli derived from tetraploid and diploid species of *Triticum* and *Aegilops,* having various genomes (Gosch-Wackerle et al. 1979; our results, data not shown) indicated a possible role for the B genome in controlling this ability. Therefore, in the present study we considered which specific chromosomes or chromosomal arms of the B genome chromosomes of common wheat (*T. aestivum*) are either positively or negatively correlated with growth and morphogenesis of immature-embryo-derived scutellar callus. The effects of several alien cytoplasms on the in vitro performance of this explant were also tested.

Materials and methods

Plant material

The following aneuploid lines of *Triticum aestivum* cv 'Chinese Spring' (Sears 1954) were used: (1) ditelosomics of the B genome, i.e. lines deficient in one pair of B genome chromosomal arms, designated 1BL, 1BS etc. (L and S symbolise the long and short arms of the chromosome, respectively). Lines 2BS, 4BS and 5BS of this series are not available; (2) compensating nullisomic-tetrasomic lines of the B genome, i.e. lines deficient in one pair of homologous chromosomes but having four of another chromosome of the same homoeologous group ($2n=42$), designated N1BT1D etc. (N and T are nullisomic and tetrasomic, respectively). Lines N6AT6D and N6DT6A were used in addition; (3) tetrasomics of the B genome, i.e. lines having four of a B genome chromosome ($2n=44$), designated T1B etc. These lines were produced and kindly provided by Prof. E. R. Sears, Univ. of Missouri, Columbia, Missouri, USA.

The series of alloplasmic lines having *T. aestivum* cv 'Chris' nucleus in combination with different plasma-types was produced and kindly provided by Prof. S. S. Maan, North Dakota State University, Fargo, North Dakota, USA. The fertile alloplasmic lines were selfed while the male-sterile alloplasmic lines (with the cytoplasm of *T. timopheevii* or *A. caudata*) were back-crossed to the donor of the nucleus, i.e. cultivar 'Chris', by hand-pollination.

Establishment, maintenance and regeneration of tissue cultures from immature-embryos

Cultures were derived from the scutellum of immature-embryos basically as described by Gosch-Wackerle et al. (1979). The ca. 1 mm long embryos were excised from the caryopses about 12 days after anthesis. For the isolation of embryos, whole spikes were surface-sterilized by immersion in 70% (v/v) ethanol for 1 min and subsequently in a 30% strength commercial bleaching solution containing 0.05% detergent ('Tween 20') for 15 min. The spikes were rinsed twice with sterile distilled water. The embryos were placed with the scutellum upwards over 8 ml solidified callus-initiation medium in 50 mm Petri dishes. The latter medium (VKM) contained macro- and microelements of V47 (Binding 1974), the organic constituents of KM (Kao and Michalyuk 1975), 3% sucrose, 0.8% agar and 2 mg/l 2,4-D. Cultures were maintained at 25 °C in the dark for 3 weeks, the precociously germinated embryos were discarded and the scutellar calli were cultured for an additional 3 weeks under the same conditions. The calli were then transferred to VKM containing 1 mg/l IAA and 1 mg/l zeatin (regeneration medium) and kept in 16 h (daily) light provided by 'white' fluorescent tubes (ca. 50 $\mu E\ m^{-2}\ s^{-1}$). The regenerated plantlets were transferred to 'Jiffy' turf-pots, kept for 1 week under plastic cover in a culture-room and subsequently planted into 3 l pots in the greenhouse.

Evaluation of results

Two parameters were used for the quantitative evaluation of growth and differentition of calli in culture: (1) the rate of scutellar callus growth, expressed as the relative increase of callus fresh weight between passages. Thus, a sample of calli was cultured on initiation medium for either 15 weeks or 50 days and was weighted at 3 week intervals and every 10 days, respectively. The 'growth rate' was calculated as the fresh weight at one passage divided by the fresh weight at the former passage; (2) the differentiation of the scutellar callus, scored as the proportion of calli belonging to one of the following classes: non-differentiated callus; callus with root hairs, root primordia or roots; callus differentiating to shoot primordia either by organogenesis or embryogenesis (within this class the presence of embryoid-like structures was noted separately); callus having rather developed shoots and roots, i.e. a plantlet. Calli carrying more than one structure were included in the class that was more 'developed' in respect to regeneration (based on the observation that calli that first had roots did not develop shoots at a later stage, while those having shoot primordia had later also roots). The calli were examined 3 times: after 3 weeks, when they were transferred to fresh initiation medium; after 6 weeks, when they were transferred to regeneration medium and after 3 weeks on regeneration medium. Thus, the 'efficiency' of differentiation and regeneration was calculated as the percent of calli belonging to each class out of the number of calli that emerged from the initially inoculated embryos. The percent of calli belonging to each class was calculated for each plant (from which embryos were excised), then the mean percent was calculated for all the plants of the same line. Comparisons of means were performed by Duncan's multiple-choice test at the significance level of 5%.

Results

In vitro culture ability of scutellar calli from different aneuploid lines

When all the available nullisomic-tetrasomic lines of the B genome were examined (Table 1) we found no significant differences in the growth rate of these calli compared to the euploid 'Chinese Spring' (since N2BT2D was not available then, we included 2BL in this experiment). The examination of the growth rate of scutellar calli from ditelosomic lines of the B genome revealed that calli derived from line 6BS, i.e. the line which is ditelosomic for the short arm of chromosome 6B, were the only ones that grew consistently slower than calli derived from the euploid control (Table 2).

The emergence of primordia from the scutellar calli derived from the aneuploid lines during callus growth is summarized in Table 3. The further differentiation of these scutellar-calli on regeneration medium is shown in Fig. 1. The results and the possible effects attributed to the various B genome chromosomes on morphogenesis of the scutellar callus are summarized as follows (see also Table 7):

Chromosome 1B. Significantly fewer calli differentiated to shoot primodia and plantlets in the line 1BS than in the control line. The examination of other lines for chromosome 1B indicated that its short arm may harbour a negative effect on differentiation; when chromosomes 1B were replaced by chromosomes 1D, the regeneration ability was improved. Moreover, a double dose of the short arms of chromosome 1B, such as in the lines N1DT1B and T1B, was correlated with a decrease in regeneration ability.

Chromosome 2B. Calli from the line missing the short arms of 2B and those from the line in which chromosomes 2B were replaced by 2D differentiated to shoot primordia and regenerated plants significantly and markedly less than did calli from the euploid line. Thus, chromosome 2D obviously did not compensate for the absence of 2B. No more lines were available for chromosome 2B, therefore a more detailed analysis of

Table 1. Gain of fresh weight and growth rate of scutellar calli derived from nulli-tetrasomic lines of *Triticum aestivum* cv 'Chinese Spring'. Growth rate was calculated as fresh weight at one passage divided by fresh weight at the former passage. Fresh weight and growth rate are followed by ± SE

Aneuploid line	No. of calli	Fresh weight (g)/callus at 3 weeks	Growth rate				Fresh weight (g)/callus at 15 weeks
			at 6 weeks	at 9 weeks	at 12 weeks	at 15 weeks	
Euploid (control)	59	0.022±0.001	2.85±0.08	1.94±0.04	1.70±0.06	1.38±0.04	0.246±0.012
N1BT1D	56	0.026±0.001	3.27±0.19	2.02±0.15	1.63±0.04	1.40±0.03	0.333±0.015
2BL	38	0.015±0.001	4.97±1.12*	2.19±0.09	1.69±0.06	1.61±0.07	0.216±0.015
N3BT3D	50	0.018±0.001	2.85±0.19	1.81±0.09	1.81±0.19	1.78±0.14*	0.235±0.016
N4BT4D	34	0.024±0.001	3.03±0.15	1.92±0.08	1.96±0.21	1.60±0.08	0.385±0.041
N5BT5D	52	0.026±0.001	2.89±0.12	1.69±0.05	1.56±0.03	1.49±0.67	0.287±0.028
N7BT7D	26	0.020±0.002	3.33±0.50	1.75±0.06	1.51±0.07	1.44±0.07	0.218±0.024

* Significantly different ($P \leqq 0.05$) from control

Table 2. Gain of fresh weight and growth rate of scutellar calli derived from ditelosomic lines of *Triticum aestivum* cv 'Chinese Spring'. Growth rate was calculated as fresh weight at one passage divided by fresh weight at the former passage. Fresh weight and growth rate are followed by ± SE

Aneuploid line	No. of calli	Fresh weight (g)/callus at 3 weeks	Growth rate				Fresh weight (g)/callus at 15 weeks
			at 6 weeks	at 9 weeks	at 12 weeks	at 15 weeks	
Euploid (control)	36	0.031±0.001	2.03±0.16	2.02±0.06	1.97±0.05	1.62±0.03	0.368±0.027
1BL	64	0.015±0.001*	3.80±0.15*	2.26±0.06*	1.76±0.02	1.61±0.08	0.371±0.029
3BL	52	0.014±0.001*	4.22±0.20*	2.06±0.06	1.72±0.02	1.53±0.09	0.311±0.020
4BL	25	0.011±0.003*	5.61±0.81*	2.33±0.07*	1.82±0.08	1.66±0.02	0.440±0.071
5BL	63	0.024±0.002	2.90±0.09	1.94±0.07	1.41±0.11*	1.86±0.20	0.281±0.008
6BS	45	0.023±0.001	2.50±0.14	1.68±0.05*	1.63±0.05*	1.56±0.03	0.258±0.029
6BL	20	0.016±0.006*	5.37±2.10*	2.20±0.16	1.88±0.08	–	–
7BL	19	0.018±0.002*	3.70±0.28*	2.06±0.07	1.39±0.09*	–	–

* Significantly different ($P \leqq 0.05$) from control

Table 3. Differentiation of scutellar calli derived from B genome aneuploid lines of *Triticum aestivum* cv 'Chinese Spring'. Results of differentiation are expressed as percent of initial calli

Chromosome	Aneuploid line	No. of calli	No. of plants	Differentiation after 3 weeks on initiation medium			Differentiation after 6 weeks on initiation medium		
				Undifferentiated callus	Roots only	Shoot primordia	Undifferentiated callus	Roots only	Shoot primordia
	Euploid (control)	759	9	14.9	1.0	83.9	14.1	0.8	85.1
1B	N1BT1D	280	3	23.3	0.7	76.0	26.0	0.0	74.0
	1BL	306	6	9.6	2.5	87.9	8.2*	2.8	89.0*
	1BS	126	3	41.1*	1.1	57.8*	35.2*	1.2	63.5*
	N1DT1B	78	2	38.6*	1.4	60.0*	55.8*	1.7	42.5*
	T1B	137	3	44.3*	0.7	55.0*	29.2*	0.8	70.0
2B	N2BT2D	100	2	63.7*	2.8	33.4*	57.3*	0.7	42.0*
	2BL	170	4	42.2*	1.3	55.2*	40.9*	2.8	56.3*
3B	N3BT3D	255	3	36.9*	0.6	62.2*	21.9	0.5	61.8*
	3BL	103	5	21.7	10.9*	67.5	23.0	2.1	74.9
4B	N4BT4D	174	3	48.8*	0.4	50.1*	35.8*	2.1	62.0*
	4BL	98	4	50.1*	15.4*	34.5*	41.2*	10.1	48.6*
5B	N5BT5D	263	3	40.3*	5.2	54.1*	28.7*	0.8	70.5*
	5BL	206	3	24.6	2.1	73.3	30.1*	0.0	69.9*
6B	N6BT6A	92	2	36.3*	0.0	63.7*	26.7	3.0	70.2
	N6BT6D	144	4	29.2	0.8	70.0	17.1	0.0	82.9
	6BL	151	6	42.9*	9.4	47.7*	30.7*	5.5	63.8*
	6BS	267	7	81.3*	2.9	15.8*	77.3*	2.2	20.5*
	N6AT6B	148	3	19.7	3.0	77.3	10.4	0.0	89.6
	N6DT6B	212	5	66.2*	5.4	28.4*	59.9*	3.3	36.8*
	T6B	406	10	19.9	0.7	79.4	18.4	0.6	80.9
	N6DT6A	178	2	72.8*	3.2	26.0*	67.6*	3.2	31.2*
	N6AT6D	261	3	29.1	7.5	63.4*	22.0	3.0	75.0
7B	N7BT7D	135	2	43.6*	1.1	55.3*	56.0*	0.0	44.0*
	7BL	145	3	28.7	1.7	69.6	16.2	0.0	83.8
	7BS	182	3	16.2	3.5	80.3	13.8	0.8	85.4
	N7DT7B	153	2	17.7	1.3	81.1	7.8	2.0	90.2

* Significantly different ($P \leq 0.05$) from the control (euploid)

its role was not possible. Nevertheless, the available data indicated that the absence of the chromosomal arm 2BS caused a marked decrease in the ratio of calli that differentiated to plantlets.

Chromosome 3B. The slightly reduced differentiation to shoot primordia in the absence of arm 3BS or the whole 3B chromosome, which was observed during early culture, diminished at the final stage of regeneration. Therefore, 3B does not seem to affect the morphogenesis of scutellar calli.

Chromosome 4B. Starting from the differentiation on initiation medium until regeneration, calli derived from the line missing the short arms of this chromosome and from the line in which 4B was replaced by 4D showed a slight but significant reduction in differentiation to shoots. Therefore, at least the short arm of chromosome 4B may slightly affect shoot regeneration.

Chromosome 5B. Absence of the chromosomal arms 5BS and the substitution of 5B by 5D resulted in a slight but statistically significant reduction in differentiation to shoot primordia and later to shoots. Thus, the short arm of this chromosome may have a minor effect on morphogenesis.

Chromosome 6B. Most calli which were derived from the line missing the long arms of chromosome 6B (i.e. line 6BS) failed to differentiate shoots and regenerate plants, while the differentiation to shoots of calli from the line lacking the short arms of chromosome 6B was reduced to a lesser degree. The analysis of additional lines in this homoeologous group revealed a more complex situation: the absence of chromosome 6B (or

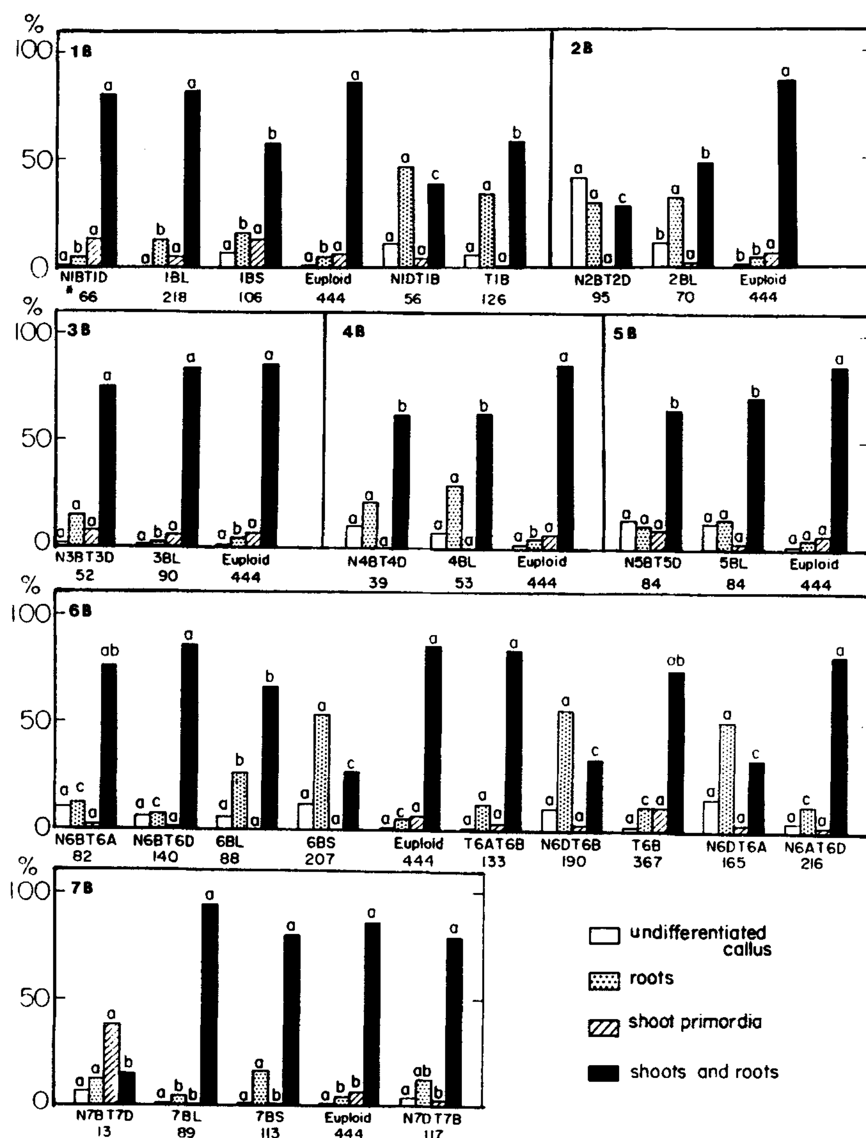

Fig. 1. Differentiation of scutellar calli derived from aneuploid lines of *Triticum aestivum* cv 'Chinese Spring' after 6 weeks on initiation medium then 3 weeks on differentiation medium. Results of differentiation are expressed as percent of calli transferred to regeneration medium; means with the same letter are not significantly ($P \leq 0.05$) different. The lines within the seven homoeologous groups are ordered according to increase in the B chromosome dose. The euploid line is presented with each group of lines, serving as control for the statistical evaluation. Lines N6DT6A and N6AT6D (which are not B genome aneuploids) were added to furnish additional information, as detailed in the text. * number of calli

probably rather its long arm) could be compensated for by its homoeologous chromosomes 6A and 6D. The same was true for chromosome 6A: its absence could be completely compensated for by 6B or by 6D. On the other hand, the absence of 6D was neither compensated for by 6A nor by 6B. This led to the conclusion that chromosome 6D has a more pronounced role in differentiation. In addition, the results indicate also a possible additive effect. In line 6BS, in spite of the presence of chromosome 6D, there was a reduction of regeneration to plants. This may reflect the fact that the long arms of only two chromosomes of homoeologous group 6 were available. On the other hand, an pair of chromosomes of this homoeologous group (as in line T6B) did not elevate the proportion of calli that regenerated plantlets above that of calli derived from the euploid line.

Chromosome 7B. The presence or absence of either arm of this chromosome did not result in any changes in the morphogenesis of the scutellar calli. Line N7BT7D that showed a marked reduction in regeneration ability was represented here by a small sample, but the results of an additional experiment under similar conditions (data not shown) did not show any effect on callus differentiation for this substitution.

The chromosomal constitution was also correlated to the pathway of differentiation 'chosen' by the scutellar calli for regeneration, i.e. whether regeneration was manifested via organogenesis or via embryogenesis. Though the appearane of embryoids is considerably influenced by culture conditions, our data show a genetic component in the determination of the morphogenetic pathway (Table 4). After 3 weeks on initiation medium, significantly more calli from line T6B devel-

Table 4. Embryoid-like structures on scutellar calli derived from B genome aneuploid lines of *Triticum aestivum* cv 'Chinese Spring'. Results of embryoid formation are expressed as percent of initial calli

Chromosome	Aneuploid line	Culture on initiation medium				Condition 3 weeks after transfer to regeneration medium		
		No. of calli	No. of plants	Calli with embryoids after 3 weeks	Calli with embryoids after 6 weeks	No. of calli	Calli with embryoids only	Calli retained embryoids after shoot regeneration
	Euploid (control)	759	9	8.9	26.1	444	1.4	25.8
1B	N1BT1D	280	3	0.0	1.4*	66	12.2	12.7
	1BL	306	6	20.6	34.6	218	4.2	38.9
	1BS	126	3	4.1	22.7	106	12.6	25.1
	N1DT1B	78	2	1.8	6.9	56	3.6	0.0*
	T1B	137	3	13.0	8.3	126	1.0	5.3*
2B	N2BT2D	100	2	0.7	0.0*	95	0.0	0.0*
	2BL	170	4	1.3	9.4	70	1.4	7.9*
3B	N3BT3D	255	3	1.4	19.0	52	0.0	41.7*
	3BL	103	5	6.3	10.0	90	6.1	13.7
4B	N4BT4D	174	3	0.0	2.6*	39	0.0	0.0*
	4BL	98	4	0.0	0.0*	53	0.0	0.0*
5B	N5BT5D	263	3	0.0	0.4*	84	2.4	2.4*
	5BL	206	3	18.4	24.0	84	2.4	17.2
6B	N6BT6A	92	2	14.1	8.6	82	2.3	11.4
	N6BT6D	144	4	7.3	8.6	140	0.8	17.9
	6BL	151	6	2.0	8.6	88	0.0	2.1*
	6BS	267	7	0.0	1.2*	207	0.5	0.0*
	N6AT6B	148	3	3.6	8.4	133	3.5	6.0*
	N6DT6B	212	5	4.4	8.1	190	0.3	5.1*
	T6B	406	10	40.0*	48.2*	367	9.4	33.5
7B	N7BT7D	135	2	4.8	6.0*	13	38.5*	7.7*
	7BL	145	3	0.8	1.6*	89	0.0	2.9*
	7BS	182	3	14.7	13.7	113	0.7	9.4*
	N7DT7B	153	2	1.4	11.2	117	1.9	20.6

* Significantly different ($P \leq 0.05$) from the control (euploid)

oped embryoids than calli of the euploid line. After 6 weeks in culture, line T6B was still outstanding in the abundance of calli that carried embryoids. At the other extreme were calli of several lines on which embryoids rarely occurred or were never observed at all (lines N1BT1D, N2BT2D, N4BT4D, 4BL, N5BT5D, 6BS and 7BL). Embryoids were still observed on two groups of calli 3 weeks after the calli were transferred to regeneration medium. One group contained calli that did not regenerate plantlets but still retained embryoids. There was no apparent influence of any of the aneuploid lines on the frequency of calli belonging to this group. The other group was comprised of calli that did regenerate plantlets and still retained embryoids. Line N3BT3D produced the highest proportion of calli of the latter type. At this stage, significantly less calli from most of the other lines were carrying embryoids, compared to calli of the control line.

In vitro culture ability of scutellar calli from different alloplasmic lines

Five lines were used, all having a *T. aestivum* cv 'Chris' nuclear genome. Four of them were alloplasmic lines with alien cytoplasms derived from various species of *Triticum* and *Aegilops* and the fifth had the cytoplasm of a different variety ('Chinese Spring') of common wheat. The tested lines have the plasma-types B, G, D, C and Mt that represent different phylogenetic distances from plasma-type B of *T. aestivum*. The examination of the growth rate of calli from lines having plasma-types B, G, C and Mt (Table 5) indicated that none of these cytoplasms had an effect on this parameter as compared to scutellar calli derived from the euplasmic control line. Similarly, when calli from the line with *Aegilops squarrosa* cytoplasm were analysed in a separate experiment (data not shown) these calli

Table 5. Gain of fresh weight and growth rate of scutellar calli derived from *Triticum aestivum* cv 'Chris' and its alloplasmic lines. Fresh weight and growth rate are followed by ± SE. *T, Triticum; A, Aegilops*

Cytoplasm donor	Plasma-type	No. of calli	Fresh weight (g)/callus at 20 days	Growth rate at 30 days	Growth rate at 40 days	Growth rate at 50 days	Fresh weight (g)/callus at 50 days
T. aestivum (control)	B (euplasmic)	338	0.022±0.001	1.85±0.09	1.48±0.02	1.45±0.03	0.079±0.005
T. dicoccum	B	312	0.038±0.002	1.76±0.06	1.47±0.02	1.48±0.03	0.079±0.005
T. timopheevii	G	48	0.047±0.007	1.92±0.17	1.64±0.09	1.35±0.05	0.098±0.011
A. caudata	C	68	0.046±0.007	1.92±0.27	1.56±0.07	1.43±0.06	0.096±0.014
A. mutica	Mt	233	0.035±0.002	1.76±0.09	1.64±0.05	1.33±0.05	0.079±0.006

Table 6. Differentiation of scutellar calli derived from *Triticum aestivum* cv 'Chris' and its alloplasmic lines. Results of differentiation are expressed as percent of initial calli or of number of calli transferred to regeneration medium

Cytoplasm donor	Plasma-type	Initial number of calli	After 3 weeks on initiation medium			After 6 weeks on initiation medium then 3 weeks on regeneration medium				
			Undifferentiated callus	Roots only	Shoot primordia	Number of calli	Undifferentiated callus	Shoot primordia	Roots only	Shoots and roots
T. aestivum (control)	B (euplasmic)	82	52.4	0.0	45.1	55	14.5	0.0	27.2	58.0
A. squarrosa	D	64	53.1	1.6	45.3	55	7.5	1.8	23.1	69.3
T. timopheevii	G	38	34.2	0.0	65.8	36	2.8	8.3*	2.8*	85.5*

* Significantly different ($P \leqq 0.05$) from the control

had similar growth rates to those of normal *T. aestivum*. Two alloplasmic lines, having the cytoplasms of *Triticum timopheevii* (type-G) and *A. squarrosa* (type-D), were tested for root and shoot differentiation from scutellar callus (Table 6). Starting from the first check after 3 weeks on initiation medium, calli derived from the alloplasmic line having *T. timopheevii* cytoplasm had a higher rate of differentiation to shoot-primordia compared to the euplasmic control line. On the other hand, the other alloplasmic line with the *A. squarrosa* cytoplasm was similar to the control. After 6 weeks on initiation medium, as is often observed, some shoot dedifferentiation occurred, but after transfer to regeneration medium, significantly more calli of the line having *T. timopheevii* cytoplasm regenerated plantlets than did the control line. Furthermore, a significantly higher proportion of calli of this alloplasmic line still retained shoot-primordia at this stage and only a few calli differentiated to roots only, indicating an even higher potential of regeneration than scored. The ratio of calli that regenerated plantlets from the line having *A. squarrosa* cytoplasm was not different from that in the control line.

Discussion

In this study we utilized aneuploid lines of common wheat to single out specific chromosomes and chromosomal arms, whose presence (or absence) affects the culture ability of immature-embryo-derived scutellar callus.

The effect of the different B genome chromosomal arms on growth and regeneration ability is summarized in Table 7. Absence of chromosomal arm 6BL was correlated to a reduced growth rate of the callus. The absence of chromosomal arms of chromosomes 1, 2, 4, 5 and 6 of the B genome was correlated to various degrees of alterations in the differentiation performance of the scutellar callus. The prominent effects of the absence of chromosome 6D, of the long arm of chromosome 6B and of the short arm of chromosome 2B on the culture ability of scutellar-callus or callus from other explant sources for wheat cell culture have not been reported before (Shimada and Makino 1975; Baroncelli et al. 1978). In this study we observed that the absence of chromosome 4B affected culture ability to a lesser extent than the former chromosomes. The

Table 7. Effect of the different B genome chromosomal arms on growth and regeneration ability of scutellar calli from common wheat (cv 'Chinese Spring')

Chromosomal arm	Callus growth	Regeneration of plantlets	Remarks
1BS	no effect	suppression	
1BL	no effect	promotion	
2BS	no effect	strong promotion	line not available
2BL	no effect	?	
3BS	no effect	no effect	
3BL	no effect	no effect	
4BS	no effect	weak promotion	line not available
4BL	no effect	?	
5BS	no effect	weak promotion	line not available
5BL	no effect	?	
6BS	no effect	weak promotion	
6BL	promotion	strong promotion	chromosome 6D is essential
7BS	no effect	no effect	
7BL	no effect	no effect	

role of chromosome 4B (especially its long arm) in promoting growth and regeneration of wheat calli has been suggested (Baroncelli et al. 1978; Mathias and Fukui 1986).

The effects that were revealed may also reflect indirect influences that affect the condition of the explant source, but they may just as well be caused by direct changes in the potential of the callus itself. In the latter case, the identification of chromosomal arms that specifically influence culture ability may lead to allocation of genes that contribute to the control of this potential.

The presented results indicate that culture ability may be correlated to or be controlled by genes carried on specific chromosomal arms of the B genome, since there was no compensation for their absence by their homoeologous chromosomes. On the other hand, it is possible that more chromosomal arms are involved in the determination of culture ability than were actually detected here, since the absence of some chromosomal arms can be compensated for by their homoeologues. Thus, some influences may not specifically be correlated to this genome.

A series of alloplasmic lines was exploited to study the influence of specific combinations of nucleus and cytoplasm on the culture ability of scutellar calli. The change in morphogenetic capability of these calli, which was correlated to the introduction of an alien cytoplasm, is in accord with numerous other reports on morphogenetic changes caused by novel nuclear-cytoplasmic combinations in *Triticum* and *Aegilops* (e.g. Maan 1979). Specifically, the *Triticum timopheevii* cytoplasm that significantly enhanced the regeneration of the scutellar callus in the present study, has been reported to have a favourable effect on embryo production in androgenesis of Triticale (Charmet and Bernard 1984) and on the frequency of callus initiation in anther culture of wheat (Picard et al. 1978). On the other hand, recently it was reported that *T. timopheevii* cytoplasm combined with the nucleus of *T. aestivum* cv 'Chinese Spring' did not have any positive effect on any culture-cycle component of scutellar callus (Mathias et al. 1986). The latter result emphasizes the importance of the specificity of the combination of a given nucleus with a certain cytoplasm in the determination of this ability.

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