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Control of tissue culture response in wheat (*Triticum aestivum* L.)

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Summary. The ability of immature embryos of wheat (*Triticum aestivum* L.) to respond to tissue culture has been shown to involve the group 2 chromosomes. The available group 2 ditelosomic and nullisomic-tetrasomic lines of 'Chinese Spring' wheat were used to determine the chromosome arm location and chromosome dosage effect associated with the expression of tissue culture response (TCR). Significant differences were found between the aneuploid lines and the euploid control for the expression of both regenerable callus formation and callus growth rate. A model is proposed suggesting that a major TCR gene is located on *2DL* and that *2AL* and *2BS* possess minor TCR genes. Furthermore, a major regulatory gene controlling the expression of TCR genes may be located on chromosome *2BL*.

Key words: Tissue culture – Callus – Wheat – Ditelosomics – Nullisomic-tetrasomics

Introduction

In common bread wheat, *Triticum aestivum* L. (2n = 6x = 42), callus induction, maintenance, and regeneration from explants of immature embryos are genotype dependent (Sears and Deckard 1982; Maddock et al. 1983; Lazar et al. 1983; Mathias and Simpson 1986). Sears and Deckard (1982) cultured immature embryos from 39 genotypes of winter wheat and identified selection ND7532 as being highly regenerable. It showed a higher percentage of calli induced and regenerable calli formed, faster in vitro growth rates, and a higher frequency of totipotent calli than the other genotypes. Analyses of crosses between ND7532 and cultivars showing intermediate-to-low responsiveness indicated a qualitative mode of inheritance for callus growth rate and plant regeneration (Salman 1986). Some crosses defined one or possibly two dominant genes, whereas other crosses indicated that major genes and additional modifier genes were involved. ND7532 was further studied using monosomic analysis, and the homoeologous group 2 chromosomes, in particular chromosome *2D*, contained genetic factors promoting regenerable callus formation and callus growth rate (Kaleikau 1988). Analysis of immature embryo cultures derived from various *4B* chromosome substitution lines has implicated the homoeologous group 4 chromosomes, specifically chromosome *4B*, as influencing regenerative capacity (Mathias and Fukui 1986; Higgins and Mathias 1987).

Interestingly, the chromosomal locations of ten of the dwarfing genes mapped to date involve only the group 2 and group 4 chromosome (Gale and Youseffian 1985). Dwarfing genes are known to have a major effect on plant growth and morphology, by affecting cellular gibberellic acid (GA) and indoleacetic acid (IAA) metabolism (Gale and Youseffian 1985). In addition, the group 2 chromosomes possess homoeoallelic loci for photoperiodism, which influence the response of wheat plants to day length (Welsh et al. 1973; Scarth and Law 1983; Scarth and Law 1984). The possibility of allelic variation at these loci may suggest that genotypic variation for callus initiation, maintenance, and regeneration involves a modification in cellular hormone metabolism (Mathias and Fukui 1986; Higgins and Mathias 1987; Kaleikau 1988). In addition to physiological factors influencing regenerability, there appears to be an important environmental component. Galiba et al. (1986) measured the in vitro response of a complete series of 'Chinese Spring'/'Cheyenne' substitution lines grown in an environmental chamber, and identified chromosomes *2D* and *4B* as well as five other chromosomes as being criti-

cal for plant regeneration. When these same lines were grown in the field, other chromosomes were implicated, however, chromosomes *7B, 7D,* and *1D* were manifested in both environments, suggesting that these three chromosomes possess genes controlling tissue culture response. Analysis of immature embryo cultures from wheat/rye addition lines indicates that rye chromosomes 6 and 7 either contain positive factors for regeneration or alleviate the effects of negative genes located elsewhere in the wheat genome (Lazar et al. 1987).

Ditelosomic and nullisomic-tetrasomic lines for a specific homoeologous group can reveal information about the chromosomal location and genetic control of genes affecting a particular character. Ditelosomic lines can be utilized in identifying the arm location of the gene being investigated, because the absence of a particular phenotype implies that the gene determining that phenotype is located in the missing chromosome arm (McIntosh 1987). Analysis of nullisomic-tetrasomic lines allows a means of comparing the effects of wheat homoeologues, in which the absence of a chromosome is compensated by the increased dosage of one of its homoeologues (Sears 1966). Utilizing ditelosomics, nullisomic-tetrasomics, and tetrasomics, Felsenburg et al. (1987) reported *2BS* to be very important for differentiation of shoots and *6BL* important in callus growth.

The purpose of this study was to analyze further the chromosomal effect reported by Kaleikau (1988) by culturing immature embryos from the available group 2 ditelosomic and nullisomic-tetrasomic lines of 'Chinese Spring' wheat and by determining the chromosome arm location and chromosome dosage effect associated with the expression of regenerable callus formation and callus growth rate.

Materials and methods

The ditelosomic (dt-) and nullisomic-tetrasomic (n-t) lines from the group 2 chromosomes of *Triticum aestivum* L. (2n = 6x = 42) cv 'Chinese Spring' (CS) were used: *dt-2BL, dt-2DL, dt-2AS, dt-2DS, n2A-t2B, n2A-t2D, n2B-t2A, n2B-t2D. n2D-t2A,* and *n2D-t2B.* These lines, originally produced by Dr. E.R. Sears, University of Missouri, USA, were provided by the Wheat Genetics Resource Center, Kansas State University, USA. Euploid CS was used as control.

Five seeds from each line were sown on moist filter paper in petri dishes and allowed to germinate in the dark. Germinated seedlings were transferred to a soil, and, and peat mix and grown to maturity under greenhouse conditions. The heads of each plant were bagged before flowering to assure self-pollinated seed. Immature seeds 12–14 days after anthesis were sampled from each of the lines. Seeds were surface sterilized, and the immature embryos were excised and placed scutellar side up on Modified Murashige and Skoog medium (MMS) containing 1.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), as described by Sears and Deckard (1982). Calli were subcultured three more times at 30-day intervals on MMS medium containing 0.75, 0.50, and 0.25 mg/l 2,4-D, respectively, for a total of 120 days. All cultures were grown in a Percival growth chamber set at 23°–24 °C and 16-h day/8-h night photoperiod (1,500 lx).

The method of Kaleikau (1988) was used for evaluating the response of immature embryo explants of wheat. Briefly, two measurements were determined. (1) mean gram fresh weight of callus growth, and (2) mean score of regenerable callus formation. Callus growth was measured by aseptically weighing callus on a balance at 60, 90, and 120 days. Regenerable callus formation was determined by scoring each callus on a scale of 1 (excellent) to 9 (poor) at 30, 60, 90, and 120 days. Scores 1, 2, or 3 represented a range of highly regenerable calli, characterized as yellow-white in color, and embryogenic, with varying degrees of green shoot primordia covering the surface. Intermediate scores 4 or 5 represented a range of embryogenic/nonembryogenic calli, off-white in color and lacking green shoot primordia. Scores 6, 7, 8, or 9 represented a range of nonembryogenic calli, light to dark brown in color, with no green shoot primordia.

The data were analyzed as an unbalanced, completely randomized design with at least ten replications per ditelo, nulli-tetra, and euploid CS line. The experimental unit (replication) was considered as one 100 × 25 mm petri plate containing approximately 50 ml of medium and five subsamples (callus). Subsamples were placed in the same relative position on fresh media at each 30-day subculture. The average of subsamples per experimental unit was used in computing the response variable for mean scores and mean callus growth rates. Statistical Analysis Systems (SAS) were used to compute pairwise *t*-tests at $P \leq 0.05$, 0.01, and 0.001 levels of significance.

Table 1. Mean score for regenerable callus formation at 30, 60, 90, and 120 days for 'Chinese Spring' (CS) group 2 ditelos (dt-)[a]

Line	No. days in culture			
	30	60	90	120
CS *dt-2BL*	3.43 ± 0.13	3.61 ± 0.27	3.50 ± 0.38	3.77 ± 0.41
CS *dt-2DL*	3.41 + 0.12	3.20 ± 0.25	2.48 ± 0.36	2.36 ± 0.39*
CS *dt-2AS*	4.38 ± 0.15***	4.83 ± 0.30***	5.51 ± 0.43***	5.97 ± 0.46***
CS *dt-2DS*	4.44 ± 0.13***	5.12 ± 0.27***	5.43 ± 0.39***	5.41 ± 0.41**
CS euploid (control)	3.21 ± 0.11	3.24 ± 0.22	2.84 ± 0.32	3.49 ± 0.46

[a] Regenerable score rating 1 = excellent to 9 = poor. Means and standard errors are from at least ten replications (five calli/rep)
*, **, *** Significantly different from the CS control within that column at $P \leq 0.05$, 0.01, and 0.001, respectively

Results

Mean response scores for calli from CS ditelosomics and the euploid control are presented (Table 1). Calli from CS *dt-2AS* and CS *dt-2DS* showed significantly poorer mean scores at each 30-day time period than the euploid CS control, clearly indicating the possibility that a related locus or loci affecting regenerable callus formation could be present on *2AL* and/or *2DL*. Calli from CS *dt-2DL* had mean scores comparable to or better than the euploid CS control at each 30-day time period, thus confirming the possibility that genes promoting regenerable callus formation are located on the long arm of *2D*. Calli from CS *dt-2BL* also had mean scores comparable to the euploid control at each 30-day time period. This may indicate differences in the 'potency' of the CS alleles controlling regenerable callus formation on *2BL* and *2DL*.

Mean callus growth rates for CS ditelosomics and the euploid control are presented (Table 2). With the exception of CS *dt-2BL* at 120 days, callus growth rates followed by same trend as regenerable callus formation. Calli from CS *dt-2AS* and CS *dt-2DS* showed significantly lower growth rates than the euploid CS control, while calli from CS *dt-2BL* and CS *dt-2DL* had growth rates equivalent to the control.

Mean response scores for calli from CS nullisomic-tetrasomics and the euploid control are presented (Table 3). The increased dosage of chromosomes *2A* and *2D* was not sufficient to compensate for the absence of *2B*. Calli from CS *n2B-t2A* and CS *n2B-t2D* showed gradually poorer scores at each 30-day time period. Significantly lower mean scores also were found for CS *n2A-t2D*, indicating that the increased dosage of chromosome *2D* was not capable of compensating for the absence of chromosome *2A*. The best compensation was found for CS *n2A-t2B*, because calli had mean scores equivalent to the euploid CS control at 60, 90, and 120 days, indicating that chromosome *2B* compensates for the absence of *2A*. Partial-to-poor compensation was found for CS *2nD-t2A* and CS *n2D-t2B*. Scores for these calli remained relatively constant or became better at 120 days, indicating that the increased dosage of chromosomes *2A* and *2B* may compensate for the absence of *2D*.

Mean callus growth rates for CS nullisomic-tetrasomics and the euploid control are presented (Table 4). In general, callus growth rates followed the same trend as those for regenerable callus formation. Calli from CS *n2A-t2B* showed an increase in growth rate equivalent to the euploid control at later subcultures, indicating that the increased dosage of chromosome *2B* sufficiently compensates for the absence of *2A*. A poor compensation was found for CS *n2A-t2D*, *n2B-t2A*, and *n2B-t2D*. Calli showed significantly lower growth rates than the euploid control at all 30-day subcultures, indicating that the increased dosage of chromosome *2D* could not compensate for the absence of *2A* and *2B*, whereas that of chromosome *2A* could not sufficiently compensate for the absence of *2B*. Increased dosage of CS chromosomes *2A* and *2B* may partially compensate for the absence of *2D* in later subcultures, since 120-day-old calli appeared to be resuming a growth rate similar to the euploid control.

Table 2. Mean callus growth rate at 60, 90, and 120, days for 'Chinese Spring' (CS) group 2 ditelos (dt-)[a]

Line	No. days in culture		
	60	90	120
Cs *dt-2BL*	0.30±0.04	0.79±0.11	0.93±0.12*
CS *dt-2DL*	0.34±0.04	0.94±0.11	1.32±0.12
CS *dt-2AS*	0.08±0.05**	0.17±0.13***	0.43±0.14***
CS *dt-2DS*	0.08±0.04***	0.22±0.11***	0.83±0.12*
CS euploid (control)	0.26±0.03	0.79±0.12	1.28±0.13

[a] Means±SE from at least ten replications (five calli/rep)
*, **, *** Significantly different from the CS control within that column at $P \leq 0.05$, 0.01, and 0.001 respectively

Table 3. Mean score for regenerable callus formation at 30, 60, 90, and 120 days for 'Chinese Spring' (CS) group 2 nulli-tetras (n-t)[a]

Line	No. days in culture			
	30	60	90	120
CS *n2A-t2B*	3.98±0.12***	3.37±0.24	2.87±0.35	3.38±0.38
CS *n2A-t2D*	4.66±0.17***	4.99±0.35***	6.89±0.51***	7.48±0.54***
CS *n2B-t2A*	5.01±0.13***	6.22±0.27***	7.53±0.39***	7.93±0.41***
CS *n2B-t2D*	5.42±0.16***	6.07±0.33***	7.32±0.48***	7.83±0.51***
CS *n2D-t2A*	4.08±0.10***	3.73±0.21	3.96±0.31**	3.74±0.32
CS *n2D-t2B*	5.99±0.20***	6.00±0.41***	6.01±0.59***	4.29±0.69
CS euploid (control)	3.21±0.11	3.24±0.22	2.84±0.32	3.49±0.46

[a] Regenerable score rating 1=excellent to 9=poor. Means and standard errors are from at least ten replications (five calli/rep)
, * Significantly different from the CS control within that column at P±0.01 and 0.001, respectively

Table 4. Mean callus growth rate at 60, 90, and 120 days for 'Chinese Spring' (CS) group 2 nulli-tetras (n-t)[a]

Line	No. days in culture		
	60	90	120
CS *n2A-t2B*	0.14±0.04**	0.67±0.10	1.11±0.11
CS *n2A-t2D*	0.05±0.05***	0.10±0.13***	0.36±0.16***
CS *n2B-t2A*	0.04±0.04***	0.04±0.10***	0.24±0.12***
CS *n2B-t2D*	0.06±0.04***	0.10±0.12***	0.41±0.15***
CS *n2D-t2A*	0.20±0.03	0.43±0.07**	0.99±0.10*
CS *n2D-t2B*	0.03±0.06***	0.16±0.15***	0.87±0.21*
CS euploid (control)	0.26±0.03	0.79±0.12	1.28±0.13

[a] Means±SE from at least ten replications (five calli/rep)
*, **, *** Significantly different from the CS control within that column at $P \leq 0.05$, 0.01, and 0.001, respectively

Discussion

Immature embryo explants from the available group 2 ditelosomics and nullisomic-tetrasomics of 'Chinese Spring' (CS) were capable of initiating callus from scutellar tissue. Significant differences were found between the aneuploid lines and the euploid control for the expression of both regenerable callus formation and callus growth rate. Since all genetic backgrounds were identical, these differences can be accounted for by the absence or presence of a critical chromosome arm and the dosage effect of homoeologous chromosomes.

In a previous study (Kaleikau 1988), it was reported that chromosome *2D* of 'ND7532' possesses genetic factors promoting the growth and regeneration of wheat callus cultures. In the present CS ditelosomic study, calli having a deficiency for the short arm of chromosome *2D* (CS *dt-2DL*) had regeneration responses and growth rates equivalent to or better than the euploid CS, whereas calli having a deficiency for the long arm of chromosome *2D* (CS *dt-2DS*) showed a significant reduction in the capacity to regenerate and grow. These results confirm the previous report of Kaleikau (1988) and further suggest that the long arm of chromosome *2D* has a major gene or genes controlling TCR. The involvement of chromosome *2D* in shoot regeneration ability from immature embryo-derived callus cultures (Galiba et al. 1986) and anther cultures (Szakacs et al. 1988) of wheat has been reported.

The CS ditelosomic study also indicated that calli deficient for the long arm of chromosome *2A* (CS *dt-2AS*) had significantly poorer regeneration responses and lower growth rates than the euploid CS. Kaleikau (1988) reported that chromosome *2A* of 'ND7532' was associated with having an effect on callus growth rate. That report and the results of the present ditelosomic study suggest that, in addition to *2DL*, the long arm of chromosome *2A* carries a locus or loci influencing the growth and regeneration ability of callus cells.

A previous study (Sears and Sears 1978) suggested that the chromosome arms *2AL*, *2BS*, and *2DL* are homoeologous. Calli deficient for the short arm of chromosome *2B* (CS *dt-2BL*) showed a subtle decrease in growth rate relative to euploid CS. This was probably due to a loss in totipotency as a result of deficiency of *2BS* carrying a locus or loci homoeologous to TCR genes found on *2AL* and *2DL* and which are necessary for the long-term maintenance of callus cultures. The simplest genetic model explaining these results is a set of homoeologous loci sensitive to in vitro culture located on chromosome arms *2AL*, *2BS*, and *2DL* of CS. Felsenburg et al. (1987) reported the presence of *2BS* as essential for shoot differentiation in tissue cultures.

Analysis of CS nullisomic-tetrasomic lines implicates all three homoeologous chromosomes as possessing genes influencing TCR. The most striking result was that found for chromosome *2B*. The increased dosage of chromosomes *2A* and *2D* could not compensate for the absence of *2B*. Furthermore, tetrasome *2B* was capable of promoting normal callus growth and regeneration in the absence of chromosome *2A* and, to a lesser extent, in the absence of *2D* at 120 days. This may suggest that chromosome *2B* of CS carriers a more potent homoeoallele, possibly a major regulator, for TCR. The involvement of a gene or genes controlling TCR on chromosome *2B* of 'ND7532' was implicated in a previous study (Kaleikau 1988). This variation in response between the group 2 chromosomes of CS and 'ND7532' may reflect allelic variation at the locus on *2B* and *2D* and/or modifying effects due to the background genotype. Higgins and Mathias (1987) cultured immature embryos from various *4B* substitution lines and found allelic variation for TCR. Modifying factors enhancing or reducing the expression of TCR have been previously suggested for wheat (Salman 1986; Kaleikau 1988) and maize (Hodges et al. 1986).

The present study has established that there is a complex relationship between the TCR genes present on each of the three homoeologous group 2 chromosomes. It is possible that this relationship may involve regulatory factors. The existence of non homoeologous regulators located far from the structural gene have been previously suggested in wheat for phosphodiesterases (Wolf et al. 1977) and storage proteins (Brown and Flavell 1981). Duplicate or triplicate sets of homoeologous regulators have also been reported for wheat proteins (Colas des Francs and Thiellement 1985; Thiellement et al. 1986). Based upon ditelo and nulli-tetra analysis, a hypothetical model was developed for the control of TCR by the group 2 chromosomes of CS (Fig. 1). It is proposed that a major TCR gene is located on *2DL* and that *2AL* and *2BS* possess minor TCR genes. Furthermore, it is likely

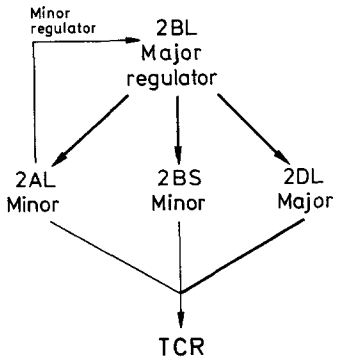

Fig. 1. Hypothesized schematic diagramm showing the control of tissue culture response (TCR) by the group 2 chromosomes of 'Chinese Spring'

that a major regulatory gene located on chromosome *2B* controls the expression of the TCR genes. The loss of TCR in the absence of *2B* (*n2B-t2A* and *n2B-t2D*) and, to a lesser extent, in the absence of *2D* (*n2D-t2A* and *n2D-t2B*) supports this hypothesis. Monosomic and substitution analysis have implicated both *2B* and *2D* (Kaleikau 1988). The poor response of *n2A-t2D* indicates that *2A* may have a minor regulatory function.

Many different chromosomes have been implicated in the TCR of wheat (Galiba et al. 1986; Mathias and Fukui 1986; Higgins and Mathias 1987; Felsenburg et al. 1987; Szakacs et al. 1988; Kaleikau 1988). It is possible that many of these chromosomes identify the location of regulatory genes which serve to modify the expression of TCR. The present studies and monosomic analysis (Kaleikau 1988) have demonstrated the involvement of the group 2 chromosomes. It is proposed that the group 2 chromosomes have both structural and regulatory roles in the TCR of wheat.

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