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Monosomic analysis 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 in cell culture was examined in crosses between the 'Wichita' monosomic series and a highly regenerable line, 'ND7532'. Segregation in disomic controls and 13 monosomic families showed a good fit to a monogenic ratio indicating a qualitative mode of inheritance. Segregation in the cross involving monosomic *2D* showed a high frequency of regeneration (93.6%) and high callus growth rate (1.87 g/90 days) indicating that *2D* is a critical chromosome. Modifying genes may be located on other chromosomes. Substitution of chromosomes from a low regenerable cultivar 'Vona' further indicated that the group 2 chromosomes, in particular chromosome *2D*, possess genetic factors promoting callus growth and regeneration.

Key words: *Triticum aestivum* – Tissue culture – Callus – Monosomic analysis – Regeneration

Introduction

Genotypic variation for tissue culture response (TCR), which includes callus initiation, growth rate, and regeneration, is evident in the major cereal crops such as wheat, *Triticum aestivum* L. (Sears and Deckard 1982; Maddock et al. 1983; Lazar et al. 1983; Mathias and Simpson 1986), corn, *Zea mays* L. (Green and Phillips 1975; Duncan et al. 1985; Hodges et al. 1986); rice, *Oryza sativa* L. (Guha-Mukherjee 1973; Fatokun and Yamada 1984; Reddy et al. 1985); barley, *Hordeum vulgare* L. (Hanzel et al. 1985); triticale, *Triticosecale* spp. L. (Nakamura and Keller 1982), sorghum, *Sorghum bicolar* L. (Ma et al. 1987), and oats, *Avena sativa* L. (Cummings et al. 1976; Rines and McCoy 1981).

Genetic studies have indicated that major genes controlling or affecting TCR are dominant or partially dominant and simply inherited. Reisch and Bingham (1980) found that bud differentiation from callus of alfalfa (*Medicago sativa* L.) is controlled by two dominant genes and that both dominant alleles must be present in order to obtain more than 75% regeneration. The genetic control of callus growth and plant regeneration from immature embryos of wheat has been shown to be dominant or partially dominant and qualitatively inherited (Salman 1986). Additionally, dominance or incomplete dominance is observed in regeneration from maize immature embryos (Hodges et al. 1986) and in tissue culture initiation from maize tassels (Rhodes et al. 1986). Likewise, Ma et al. (1987) suggest that at least two complementary dominant genes control the ability to regenerate from immature embryos of sorghum. Mathias and Atkinson (1988) suggest that the reduced height/gibberellic acid insensitivity (*Rht*) genes have significant effects on the growth and morphogenesis of calli initiated from immature embryos of wheat.

Recently, Lazar et al. (1987) cultured anthers and immature embryos from a set of seven 'Chinese Spring'/rye addition lines and concluded that rye chromosome 4 contained factor promoting anther culture response and that rye chromosomes 6 and 7 possess positive factors for immature embryo culture response. In that study, no correlation was found between anther culture and embryo culture responses, suggesting that they are controlled by different genetic systems. Henry and de Buyser (1985) made crosses with the wheat cultivar 'Aurora', which possesses a *1B/1R* translocation, and proposed that genetic factors involved with anther culture response are located on the *1RS* chromosome arm.

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Additional chromosomes have been identified for immature embryo culture. Mathias and Fukui (1986) cultured immature embryos from a line in which the *4B* chromosome of 'Chinese Spring' was substituted with the *4B* chromosome of 'Cappelle-Desprez' (*Cap4B*) and concluded that the *Cap4B* chromosome improved the performance of calli in vitro. Higgins and Mathias (1987) analyzed further the *4B* chromosomal effect, by examining substitution lines in which the 4B chromosomes of 'Chinese Spring' and 'Cappelle-Desprez' have been replaced by the *4B* chromosomes of other varieties. Significant differences were found among the lines for callus growth, shoot primordia formation, and shoot regeneration. Felsenburg et al. (1987) reported several *B* genome chromosomes to be critical for TCR. The absence of *6BL* was correlated to a reduced callus growth rate and *2BS* seemed to be essential for differentiation of shoots from callus. Galiba et al. (1986) found no differences for callus induction from immature embryos of a set of 'Chinese Spring'/'Cheyenne' substitution lines. However, differences for shoot regeneration ability were found and appeared to be affected by environmental factors. In growth chambers, chromosomes *5A, 4B, 7B, 1D, 2D, 3D,* and *7D* affected regeneration ability. In the field, chromosomes *6A, 7B, 1D, 4D, 6D,* and *7D* were critical. Because chromosomes *7B, 1D,* and *7D* were manifested in both environments, it was concluded that genetic factors affecting shoot regeneration ability in embryo-derived callus were located on these three chromosomes. At present these studies have implicated many chromosomes possessing genes controlling TCR, despite the fact that genetic studies indicate a qualitative mode of inheritance. Different genes may be involved and genetic backgrounds certainly influence expression.

The potential impact that plant biotechnology will have on crop improvement is partly dependent on the efficiency of tissue and cell culture regeneration systems. Therefore, further genetic studies are warranted to more adequately describe the nature and location of genes controlling *in vitro* development. The purpose of this study was to use monosomic analysis to determine the chromosomal location of genes controlling regenerable callus formation and callus growth rate from immature embryos of a highly regenerable wheat genotype, 'ND7532'.

Materials and Methods

The winter wheats selected for this study were 'ND7532' (ND), 'Vona' (VO), and the 'Wichita' (WI) monosomic series, except that chromosome *2B* was from 'Cheyenne' (CH). Callus derived from the scutellar tissue of immature embryos of ND and VO represent extreme ranges of expression for in vitro aptitude (Sears and Deckard 1982; Guenzi et al. 1983). ND presumably carries one or possibly two dominant genes and additional modifier genes controlling callus growth rate and plant regeneration (Salman 1986). ND consistently shows an excellent callus phenotype, which is fast growing, embryogenic, and highly regenerable (Sears and Deckard 1982). VO callus is characterized by its slow growth rate, few embryogenic regions, and a low frequency of regeneration (Guenzi et al. 1983). The WI monosomic series, originally produced by Dr. R. Morris at the University of Nebraska, USA, was obtained from the Wheat Genetics Resource Center, Kansas State University, USA.

The 20 WI monosomics and one CH monosomic *2B* were crossed as females to disomic ND. Seeds from each F_1 monosomic family were grown in the greenhouse and one or two monosomic plants per family and five disomic control plants were identified cytologically by counting 41 and 42 chromosomes, respectively, in root tip cells. All F_1 monosomic seedlings, the F_1 disomic controls, and parents ND, WI, and CH were grown in pots under greenhouse conditions. The heads of each plant were bagged before flowering to assure self-pollinated seed. Immature seeds were collected 12–14 days after anthesis from each of the parents, the segregating 21 monosomic families, and disomic controls. 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 two more times at 30-day intervals on MMS medium containing 0.75 and 0.5 mg/l 2,4-D, respectively, for a total of 90 days. All cultures were grown in a Percival growth chamber set at 23°–24 °C and 16-h day/8-h night photoperiod (1500 lx).

At the end of each 30-day subculture, two measurements were recorded. Callus growth rate was determined on a random sample of calli ($n = 5–15$) by aseptically weighing the callus on a balance. Secondly, each callus was scored (1 excellent to 9 poor) for regenerability based upon the frequency of embryogenic green sectors and/or shoots per callus, callus texture, and callus color. The following descriptive scoring scale was used: (1) Calli covered with embryogenic green sectors and/or small shoots, friable in texture, and butter colored. (2) Calli with approximately two-thirds of their surfaces covered with embryogenic green sectors and/or small shoots, friable in texture, and butter colored. (3) Calli with approximately one-third or less of their surfaces covered with embryogenic green sectors and/or small shoots, friable, and butter colored over 75% of its area but nonfriable and off-white in other regions. (4) Calli with no embryogenic green sectors or green shoots, and ratio of butter-colored friable to off-white nonfriable callus of approximately 50%. (5) Calli with no embryogenic green sectors or shoots, mostly nonfriable in texture, and entirely off-white in appearance. (6) Calli similar to score 5, except small light brown necrotic patches are beginning to appear on the callus surface. (7) Calli similar in appearance to score 6, except the entire surface is light brown with dark brown patches. (8) Calli similar in appearance to score 7, except the entire callus is dark brown in color. (9) Calli dead.

To confirm the monosomic results, genetic stocks were developed in which the identified critical ND chromosome was replaced by the homologous chromosome from the poor regenerator VO. The substitution procedure involved crossing the critical ND monosomic F_1s (mono WI × ND) as females to disomic VO. Two monosomic plants from each cross were cytologically identified by counting 41 chromosomes in root tip cells. Identified seedlings and VO controls were grown in pots under greenhouse conditions and the heads were bagged to assure self-pollinated seed. Immature embryos were cultured and the calli were evaluated for growth rate and regenerability, as previously described.

The F_2 populations from each F_1 plant within a monosomic or disomic line were statistically tested for homogeneity before pooling. The data of Salman (1986) clearly indicate that most

Table 1. Frequency of regenerable (REG) calli and mean growth rate (GR) of callus at 30, 60, and 90 days for parental genotypes 'ND7532' (ND), 'Wichita' (WI), 'Cheyenne' (CH) and 'Vona' (VO) winter wheats[a]

Genotype	No. days in culture					
	30		60		90	
	REG (%)	GR (g)	REG (%)	GR (g)	REG (%)	GR (g)
ND	95	0.08 ± 0.01	97	0.86 ± 0.11	98	2.02 ± 0.19
WI	17	0.05 ± 0.01	23	0.14 ± 0.02	23	0.35 ± 0.09
CH	32	0.04 ± 0.01	35	0.27 ± 0.04	33	0.94 ± 0.14
VO	31	0.08 ± 0.01	33	0.30 ± 0.04	28	0.64 ± 0.08

[a] 75–110 calli were scored for regenerability at each 30-day subculture for callus growth rate; mean and standard error are given

genotypes evaluated showed a good response at 30 days and then begin to shift towards either a good or bad response at 60 days, so that at 90 days intermediates seldom were present. The same general trend was found in the present study, therefore, the evaluation of regenerable callus formation and growth rate is reported for 90-day old calli maintained on MMS medium containing 0.5 mg/l 2,4-D. Calli scored 1, 2, or 3 were pooled to form the regenerable (REG) class and calli scored 4, 5, 6, 7, 8, or 9 were pooled to form the nonregenerable (NREG) class. Callus regeneration frequency was measured as the percentage of calli scored as 1, 2, or 3. Chi-square statistical analysis was used to test F_1 monosomic families segregating for REG and NREG calli. The effect of the VO chromosomes was estimated by calculating the differences between the percent regeneration frequency and mean callus growth rate for the ND chromosome and the substituted VO chromosome. Standard errors (SE) were calculated as described by Steele and Torrie (1980).

Results

The frequency of regenerable calli and growth rate of callus for parental genotypes are presented (Table 1 and Fig. 1). ND showed a higher frequency of REG calli at 30, 60, and 90 days and higher callus growth rates at 60 and 90 days compared to WI, CH, and VO. These results are consistent with previous work (Sears and Deckard 1982; Guenzi et al. 1983; Salman 1986) and indicate the extent of genotypic variability for in vitro aptitude among the parents used in this study, with ND expressing a high degree of morphogenic competence over time.

The mode of segregation in monosomic F_2 families, including the disomic control, are presented (Table 2). A good fit to a monogenic ratio of 3 REG:1 NREG was obtained in the disomic control ($p=0.54$). Based upon this result, a 3 REG:1 NREG ratio was used to test monosomic F_2 families for a noncritical cross. Monosomics of 14 families – *1A, 2A, 3A, 4A, 5A, 7A, 1B, 2B, 4B, 6B, 1D, 3D, 4D,* and *7D* – showed good fits to a 3 REG:1 NREG ratio, indicating that these chromosomes are noncritical.

Segregation ratios for each of the remaining seven monosomic families deviated from the expected 3 REG:1 NREG ratio for a noncritical chromosome. Since the F_2 disomic control fits a single gene model, the expected ratio used to test for a critical cross is 97 REG:3 NREG (Kuspira and Unrau 1959). In the cross involving *2D*, 44 calli out of 47 from the progeny of monosomic *2D* F_1 showed a high frequency of regeneration (93.6%) (Table 2). A satisfactory fit to the ratio 97 REG:3 NREG ($p=0.17$) was obtained, indicating that *2D* is a critical chromosome for regenerable callus formation.

Deviations from the expected segregation ratios for a critical or noncritical cross are generally ascribed to modifying genes (Sawhney et al. 1981; Kosner and Bartos 1982; Maan et al. 1984). Therefore, in addition to chromosome *2D*, F_2 segregation in a monosomic *7B* family deviated towards regenerability with regeneration frequency of 89.4% (Table 2), indicating the location of a modifying gene (enhancer) promoting callus regeneration. Segregation ratios in five of the monosomic families (*6A, 3B, 5B, 5D,* and *6D*) deviated towards nonregenerability, with regeneration frequencies of 57.8%, 52,4%, 44.2%, 47.1%, and 40.0%, respectively (Table 2), indicating the location of modifying genes (reducers) that cause a surplus of nonregenerable calli.

Mean gram callus growth rate for each of the F_2 monosomic families and disomic control are presented (Table 2). Chromosomes *2D* and *2B* had the highest callus growth rates, being 1.87 g for *2D* and 1.92 g for *2B*. Moreover, homoeologous chromosome *2A* also showed a high callus growth rate (1.66 g). Despite the high regeneration frequency found for chromosome *7B* (89.4%), a correspondingly high callus growth rate was not found. All noncritical chromosomes, with the exception of *5A, 6B,* and *4D*, had growth rates ≤ 1.33 g, which are similar to or lower than the callus growth rate of disomic controls (Table 2). With the exception of *6A*, chromosomes *3B, 5B, 5D,* and *6D*, identified as having modifying fac-

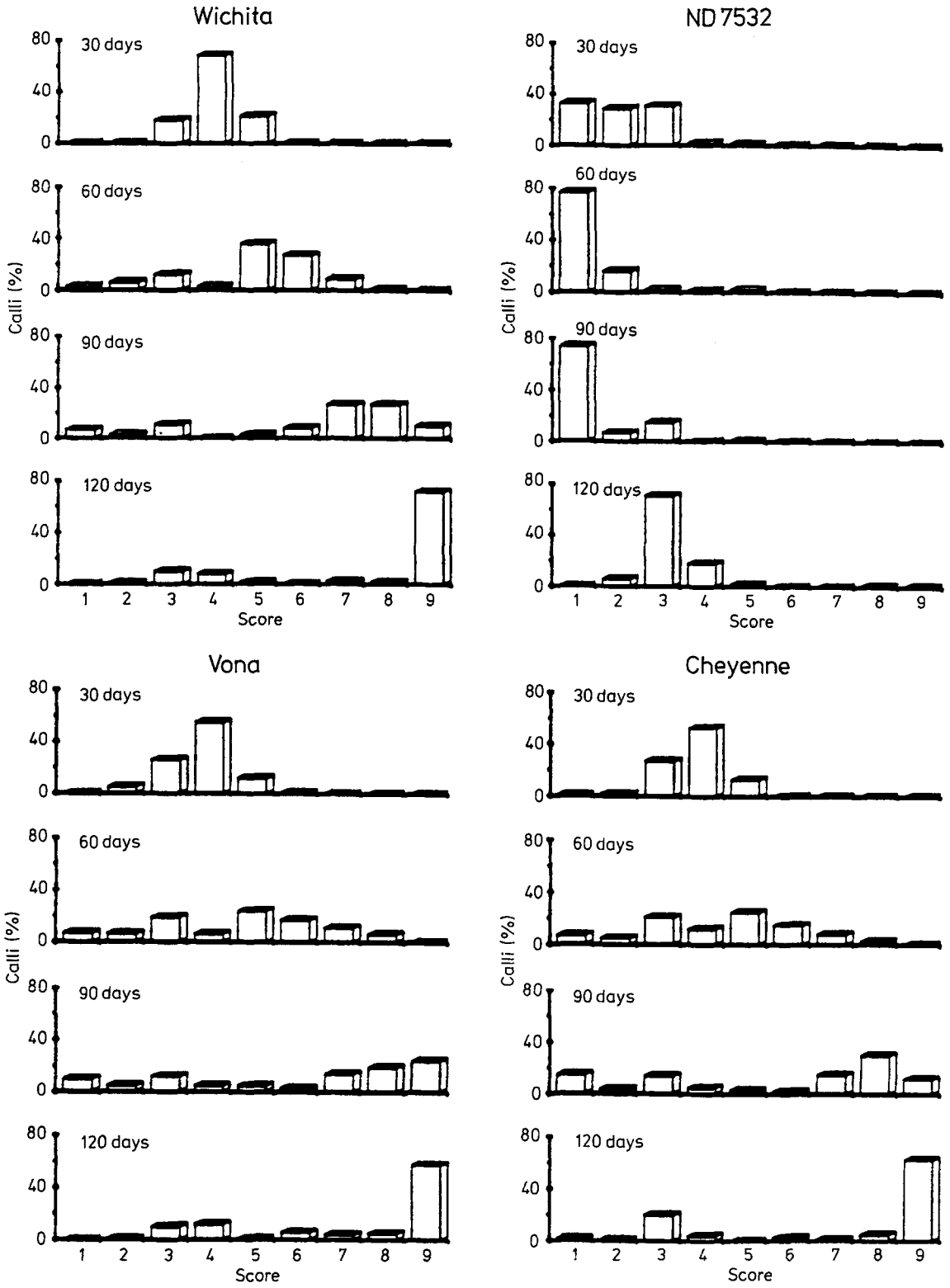

Fig. 1. Frequency distributions for parental genotypes 'Wichita', 'ND7532', 'Vona' and 'Cheyenne' at 30, 60, 90, and 120 days in culture (1 = excellent to 9 = poor).

Table 2. F_2 segregation for regenerable (REG) and nonregenerable (NREG) calli and mean growth rate (GR) of callus from crosses involving the 'Wichita' (WI) wheat monosomic series with 'ND7532' (ND)

Chromosome	No. calli				*P* value		
	REG	(%)	NREG	Total	(3:1)	(97:3)	GR (g)[a]
1A	36	(73.5)	13	49	0.80	***	0.76 ± 0.15
2A	35	(72.9)	13	48	0.74	***	1.66 ± 0.39
3A	75	(76.5)	23	98	0.73	***	1.23 ± 0.28
4A	31	(72.1)	12	43	0.66	***	0.71 ± 0.22
5A	38	(74.5)	13	51	0.93	***	1.43 ± 0.26
6A	41	(57.8)	30	71	***	***	1.19 ± 0.31
7A	38	(76.0)	12	50	0.86	***	1.30 ± 0.25
1B	40	(74.1)	14	54	0.87	***	1.30 ± 0.25
2B	48	(84.2)	9	57	0.10	***	1.92 ± 0.32
3B	11	(52.4)	10	21	*	***	0.78 ± 0.28
4B	68	(70.8)	28	96	0.65	***	1.30 ± 0.31
5B	19	(44.2)	24	43	***	***	0.61 ± 0.21
6B	51	(76.1)	16	67	0.83	***	1.61 ± 0.21
7B	42	(89.4)	5	47	*	*	1.13 ± 0.24
1D	43	(70.5)	18	61	0.58	***	1.32 ± 0.23
2D	44	(93.6)	3	47	**	0.17	1.87 ± 0.22
3D	38	(76.0)	12	50	0.86	***	1.33 ± 0.31
4D	56	(71.8)	22	78	0.52	***	1.48 ± 0.25
5D	33	(47.1)	37	70	***	***	0.89 ± 0.20
6D	34	(40.0)	51	85	***	***	0.73 ± 0.20
7D	84	(73.7)	30	114	0.74	***	1.10 ± 0.18
Disomic control[b]	76	(72.4)	29	105	0.54	***	1.30 ± 0.13

[a] Mean gram callus growth rate ± SE
[b] WI × ND
*, **, *** Significant deviation from the tested ratio at $P \leq 0.05$, 0.01, and 0.001, respectively

Table 3. Effect of substituted 'Vona' (Vo) wheat chromosomes on the frequency of regenerable (REG) calli and mean growth rate (GR) of callus

Chromosome	REG (%)			GR (g)		
	ND	VO	Difference ND–VO	ND	VO	Difference ND–VO
2A	72.9	19.6	53.3 ± 2.9	1.66	0.50	1.16 ± 0.12
7A	76.0	33.4	42.3 ± 7.2	1.30	0.54	0.77 ± 0.03
2B	84.2	24.9	59.3 ± 3.7	1.92	0.60	1.32 ± 0.12
7B	89.4	31.6	57.8 ± 0.6	1.13	0.45	0.71 ± 0.02
2D	93.6	18.8	74.8 ± 7.6	1.87	0.50	1.37 ± 0.01
7D	73.7	16.0	57.7 ± 5.7	1.10	0.32	0.78 ± 0.08
Controls (ND, VO)	98.0	28.0	70.0 ± 4.7	2.02	0.64	1.38 ± 0.08

tors inhibiting callus regeneration, showed correspondingly low callus growth rates (Table 2).

The substitution of VO chromosomes caused a marked loss in the frequency of regenerable calli and growth rate of callus (Table 3). Substitution of chromosome *2D* from VO appeared to have a relatively greater loss in percent regeneration (74.8%) compared to the other chromosomes tested, and was similar to the difference between ND and VO controls (70.0%). Furthermore, the substitution of *2A*, *2B*, and *2D* from VO caused a greater loss in callus growth rate compared to the group 7 chromosomes, with *2B* and *2D* producing a difference similar to that between ND and VO controls (1.38 g).

Discussion

The chromosomal location of genes controlling TCR from immature embryos of wheat was studied in crosses between the 'Wichita' monosomic series and a highly regenerable genotype 'ND7532'. Contrasting phenotypes for in vitro aptitude among the parents permitted the use of monosomic analysis to determine which chromosomes of 'ND7532' possess genetic factors controlling regenerable callus formation and callus growth rate.

The mode of segregation in disomic controls showed a good fit to a monogenic ratio, confirming the report of Salman (1986) who suggested that the genetic control of regenerable callus formation from immature embryos of

'ND7532' is dominant or partially dominant and simply inherited. Mathias and Fukui (1986) and Higgins and Mathias (1987) report that chromosome *4B* from Russian and European wheat cultivars has a significant effect on the regenerative capacity of callus derived from immature embryos. In this study, monosomic analysis identified chromosome *4B* as noncritical, suggesting that it is not involved in the highly regenerable response of 'ND7532'. Felsenburg et al. (1987) also reported *4B* to have a lesser effect on TCR than other chromosomes for 'Chinese Spring' aneuploid lines. One possible explanation is that 'ND7532', 'Wichita', 'Cheyenne', and 'Vona', used in this study, show a high degree of relatedness and may be homogeneous for either the presence or absence of genes controlling TCR on the group 4 chromosomes, whereas TCR in Russian and European wheat cultivars may involve the group 4 chromosomes. A relationship between germplasma source and TCR has been reported in maize (Tomes and Smith 1985; Hodges et al. 1986) and alfalfa (Brown and Atanassov 1985).

Chromosome *2D* of 'ND7532' was clearly identified as a critical chromosome possessing a locus or loci for regenerable callus formation. Salman (1986) suggested that major gene(s) and additional modifier genes may be involved in crosses between 'ND7532' (high) and 'TAM 105' (intermediate) responders. Likewise, Hodges et al. (1986) evaluated crosses between a highly regenerable maize inbred, A188, and 25 inbreds representing major maize germplasm groups. Some of the crosses to A188 did not show high levels of regeneration, which led to the suggestion that suppressors of gene expression for regeneration may exist in some maize inbreds. Modifying genes may explain the segregation ratios for some of the F_2 monosomic families which deviated from the expected ratios for a critical or noncritical cross. Modifying gene (enhancer) promoting callus regeneration may be located on chromosome *7B*, as F_2 segregation deviated towards regenerability. Segregation ratios for chromosomes *6A*, *3B*, *5B*, *5D*, and *6D* deviated towards nonregenerability, indicating the location of modifying genes (reducers) that inhibit regenerable callus formation. Recently, Lazar et al. (1987) cultured immature embryos from a set of seven 'Chinese Spring'/rye addition lines and found significant variation for regenerative capacity. Rye chromosomes 6 and 7 were identified as containing genes that either (1) positively affect the regeneration response, or (2) alleviate the effects of negative genes located elsewhere in the wheat genome. Perhaps rye chromosomes 6 and 7 relieve the effects of inhibitory genes located on wheat chromosomes *6A*, *3B*, *5B*, *5D* and/or *6D*, or modify the expression of enhancer genes located on *2D* and/or *7B*.

Chromosomes *2D* and *2B* had high callus growth rates. Interestingly, homoeologous chromosome *2A* showed a high callus growth rate as well, thus implicating the group 2 chromosomes as possessing genetic factors that control callus growth rate. All noncritical chromosomes, with the exception of *5A*, *6B*, and *4D* had growth rates similar to or lower than the disomic controls. Chromosomes *6A*, *3B*,*5B*, *5D*, and *6D*, identified as having modifying factors inhibiting callus regeneration (excluding *6A*), showed correspondingly low callus growth rates. In the broadest sense, the results may suggest partial linkage for regenerable callus formation and callus growth rate. However, considering the integrated state of cellular and developmental metabolism as well as the influence of environmental factors on in vitro development, pleiotropic gene action is a more reasonable explanation.

Because chromosomes *2D* and *7B* of 'ND7532' appeared to have genetic factors promoting callus regeneration, the group 2 and group 7 chromosomes were further studied by substituting them with homologous chromosomes from the poor regenerator, 'Vona'. The substitution of chromosome *2D* from 'Vona' showed a relatively greater loss in percent regeneration compared to the other chromosomes tested. In general, the substitution of the group 2 chromosomes of 'Vona' gave a marked loss in callus growth rate compared to the group 7 chromosomes. The results confirm that chromosome *2D* has a major influence on regeneration and growth rate of callus cells. More interestingly, chromosomes *2A* and *2B* of 'Vona' caused a marked loss in callus growth rate, thus implicating the involvement of the group 2 chromosomes on TCR.

In wheat, similar genes are frequently located at similar positions on each of the three chromosomes of a homoeologous group. Three homoeoallelic genes for photoperiodism, *Ppd1* on *2D*, *Ppd2* on *2B* and *Ppd3* and *2A* influence the response of wheat plants to day length (Welsh et al. 1973; Scarth and Law 1983, 1984). Significant differences for callus growth rate and regenerability have been demonstrated for immature embryo explants of wheat cultured under light and dark treatments (Stein et al. 1986; Mathias et al. 1986; Higgins and Mathias 1987). Studies on tobacco by Cassells et al. (1982) suggest that endogenous hormone levels may be responsible for the altered responses produced by different photoperiods, because pretreatments of donor plants in the dark for 48 h decreased the number of shoots produced from petiole explants. This was found to be correlated with an increase in endogenous indoleacetic acid (IAA) levels. It is possible that allelic variation at the *Ppd* loci alters endogenous IAA metabolism in callus cells of ND7532, thus modifying their sensitivity to exogenous 2,4-D supplied by the MMS culture medium.

Mathias and Fukui (1986) suggest that the substitution of the *Cap4B* chromosome, either through the introduction of a growth stimulating gene(s) or loss of an inhibitory gene(s), results in an increase in cell prolifera-

tion and shoot regeneration. They note that the reduced height/gibberellic acid alleles, (*Rht*), *Rht1*/*Rht3*, and *Rht2* are located on chromosomes *4A* and *4D*, respectively; and the 'grass clump dwarfness' allele *D3* is located on chromosome *4B*. This led them to hypothesize that an allele at the *D3* locus or the *4B* equivalent of the *Rht* loci accounts for improved tissue culture performance of *Cap4B* line through an effect on cellular hormone metabolism. That hypothesis does not totally contradict the results of this paper. The group 2 chromosomes have been associated with the control of GA metabolism (Gale and Law 1973). Genes *Rht8*, *D1*, and *D4* have been mapped to chromosome *2D* (Gale and Youseffian 1985; Worland and Law 1986), and *D2* homoeoallelic to *D4* is located on chromosome *2B* (Worland and Law 1986). Furthermore, there may be an *Rht* gene located on chromosome *2A* (Gale and Youseffian 1985). Perhaps allelic variation at the *Rht* and *D* loci located on the group 2 chromosomes results in altered cellular hormone metabolism. Wernicke and Milkovits (1987) suggest that changes in cell sensitivity to growth substance, and not only the concentration of these compounds, may play an important role in the responsiveness of wheat leaf explants. Trewavas (1982) maintains the view that changes in sensitivity to growth substances are directly correlated with changes in the number of receptors. It is possible that genotypic variation for tissue culture response involves a modification in the number of receptor sites on the cell membrane, causing a differential sensitivity of callus to applied growth regulators. Despite this association between dwarfing alleles and TCR, 'ND7532' is a standard height wheat; therefore, it is possible that other genes are the main effectors.

In conclusion, this study has demonstrated the involvement of the group 2 chromosomes in the superior TCR of 'ND7532' and has shown that modifying genes with lesser effects may be located on other chromosomes. The presence of major genes controlling TCR suggests that breeding and selection for genotypes amenable to tissue culture are feasible. It is proposed that morphogenetic competence is influenced by the ability of callus cells to synthesize, transport, and utilize endogenous as well as exogenous plant growth hormones/regulators.

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