Chromosomal Effects on *In Vitro* Morphogenesis in Wheat Intervarietal Substitution Lines

Nedyalka Dineva TYANKOVA, Nedyalka ZAGORSKA, Vasil CHARDAKOV, Ani DRYANOVA and Boyan DIMITROV

Institute of Genetics "Acad. D. Kostoff", Bulgarian Academy of Sciences, Sofia, Bulgaria

Abstract: The effect of individual chromosomes on *in vitro* morphogenic ability of immature embryos was studied using a Chinese Spring/Timstein substitution series. For this purpose 20 wheat lines of Chinese Spring with consequently substituted chromosome pairs from the cultivar Timstein together with the two parental cultivars were investigated. The regeneration ability of the studied lines was quantified by two parameters: frequency of regenerative calli and coefficient of propagation. The influence of the 5A, 1B and 4D chromosomes on *in vitro* morphogenesis was determined and the effect of 2A, 3A, 3B, 4B, 6B and 1D chromosomes, reported by other authors, was confirmed. The data indicated that the intervarietal substitutions of certain chromosomes caused significant variation in the number of morphogenic calli and recovered regenerants, in spite of the fact that the two parental cultivars were of similar morphogenic ability. This probably reflects the allele variation in a given locus on these chromosomes and/or background effect. The data suggest that the chromosome substitutions may have different compensatory effects depending on the similarity of the corresponding chromosomes and also on the possibilities of their expression in an alien genetic background.

Keywords: immature embryos; genetic control; callus induction; regeneration ability

The use of tissue culture for breeding and genetic manipulation is limited by insufficient knowledge of the genetic and molecular control of somatic embryogenesis and plant regeneration. In hexaploid wheat, a great number of investigations were carried out to determine the chromosome localization of genes controlling the tissue culture response (TCR) (see rev. Tyankova & Zagorska 2001). Allelic differences between genotypes were measured by means of quantitative genetics, Mendelian inheritance, QTL, substitution lines and aneuploid and translocation line analysis (Galiba *et al*. 1986; Mathias & Fukui 1986; Higgins & Mathias 1987; Felsenburg *et al*. 1987; Kaleikau *et al*. 1989; Henry *et al*. 1994a, b; Ben Amer *et al*. 1995, 1997). Wheat regeneration was found to be determined by a polygenic system (Galiba *et al*. 1986). It was stated (Mathias & Fukui 1986) that the 4B chromosome of Cappelle Desprez carried factor(s) for stimulating wheat callus growth, morphogenesis and regeneration. These conclusions were confirmed by Higgins and Mathias (1987) when studying Chinese Spring and Cappelle Desprez, in which the 4B chromosome was substituted with the homologous chromosome from other cultivars. As a result of monosomic analysis in cv. "ND 7532", Kaleikau *et al*. (1989) identified the 4B chromosome as non-critical. Felsenburg *et al*. (1987) also reported that the 4B chromosome had a lesser effect upon the *in vitro* response compared to other chromosomes of Chinese Spring. Galiba *et al*. (1986) did not report any differences regarding callus induction from immature embryos in a series of Ch. Spring/Cheyenne substituted lines. However, they found that there were certain differences in the regeneration capacity of shoots. According to them, the genetic factors influencing the shoot regeneration

capacity in callus obtained from embryos were localized on the 7B, 1D and 7D chromosomes. According to Felsenburg *et al*. (1987), several chromosomes from the B genome (i.e. $6B^L$ and $2B^S$) influence the differentiation of the scutellar callus to a different extent.

As it is evident, the genetic control of the *in vitro* response still remains not sufficiently clarified. The participation of various chromosomes in the *in vitro* control has been pointed out by different investigators and often contradictory results have been reported. Different results of the different investigators revealed the complexity of this biological process. The strong dependence of the regeneration ability on the genotype is an important factor making the genetical assessment of cereal *in vitro* response quite necessary.

In former investigations on the effect of intervarietal chromosome substitutions both parental cultivars differed in their *in vitro* response. It is interesting to know what the effect of the chromosome substitutions in cultivars that are similar in their reaction to *in vitro* cultivation is like.

In this work we studied the effect of individual chromosomes on the behaviour of wheat tissue cultures using a series of 20 substituted lines of the cv. Chinese Spring with chromosomes from the cv. Timstein. The two cultivars were of similar response to *in vitro* cultivation.

MATERIAL AND METHODS

A series of 20 wheat lines of Chinese Spring (CS) with consequently substituted chromosome pairs from the cultivar Timstein (Tms) together with the two parental cultivars with the genome formula $2n = 6x = 42$ were studied. Chinese Spring is a widely used cultivar in studies of wheat. The cultivar Timstein originates from *Triticum timopheevi* × Streinwebel (a German cultivar coming from Africa) and is resistant to stem and leaf rusts (SR11- 6BL-1AS; Lr10-1AS; L23-2BS (McIntosh *et al*. 1991). The intervarietal substitution series was released by E. Sears and the seeds were kindly provided by Prof. Watanabe, from the Faculty of Agriculture, Gifu University, Japan. The donor plants were grown under greenhouse conditions.

Immature, 14 days old embryos from the substitution lines and the parental cultivars were previously sterilized, excised under sterile conditions and cultivated in the dark at 25°C. The isolated semitransparent embryos, 1–1.2 mm long, were placed with the scutellum upwards on Murashige and Skoog (MS) (1962) nutrient medium in which the quantity of macrosalts was twofold elevated. The nutrient medium was supplemented with 150 mg/l L-asparagine, 0.4 mg/l thiamine, 2.5 mg/l 2,4 dichlorophenoxyacetic acid (2,4-D) and 60 g/l sucrose. The frequency of callus induction was recorded 3 weeks after inoculation. The vital, normally developing calli were further cultivated on MS nutrient medium supplemented with 0.5 mg/l kinetin, 0.5 mg/l α-naphthaleneacetic acid (NAA), 1 mg/l benzylaminopurine (BAP), 0.5 mg/l thiamine, 150 mg/l glutamine and 1000 mg/l casein hydrolysate for regeneration. The morphogenic ability of callus was studied after 20–25 day cultivation on the above-mentioned nutrient medium. The regeneration ability of the studied lines was quantified by two parameters: frequency of regenerative calli and coefficient of propagation (number of plants per callus). Regenerants were rooted on basal MS nutrient medium with twofold reduced quantity of macrosalts to which 0.4 mg/l $CuSO_4$ and 2 mg/l indole-3 butyric acid (IBA) were added.

The data were subjected to ANOVA followed by Student's *t*-test.

RESULTS AND DISCUSSION

The immature embryos grew fast within the first week of cultivation. Proliferating intensively, they accumulated unorganized cell mass. All twenty substitution lines involved in this study had a high potential for callogenesis (90–100%) (Table 1). This indicated that the conditions for callus induction were optimal. There was no significant variation between the different lines in relation to callus induction. The potential genotype differences were not manifested at this phase of the *in vitro* response. This observation is in agreement with those made by Galiba *et al*. (1986), Higgins and Mathias (1987) and Henry *et al*. (1994b). Most of the calli were yellowish and of granular structure. There were also soft, whitish calli of loose structure, though present rarely. The yellowish nodule-structured calli were cultivated in a regeneration nutrient medium. Some of them produced plantlets possessing both roots and shoots, and other produced only shoots. The latter were rooted on the MS medium supplemented with IBA.

A decline in the mean value of both parameters (frequency of regenerative calli and coefficient of

Table 1. Effect of chromosome substitutions on *in vitro* TCR in Chinese Spring/Timstein substitution lines

Substituted lines	Embryos tested N	Induced calli		Morphogenic calli		Calli producing regenerants		Fully developed plantlets N	Mean number of plants per regenerated callus
		N	%	N	%	N	%		
Chinese Spring (CS)	194	173	89.1	99	57.2	62	62.6	167	2.7
Timstein (Tms)	166	155	93.3	98	63.2	65	66.4	176	2.7
CS1ATms	141	132	93.6	84	36.6	37	44.0	85	2.3
CS1BTms	203	203	100.0	83	40.9*	21	25.3**	57	2.7
CS1DTms	219	219	100.0	90	41.1*	10	11.1***	30	3.0
CS2ATms	149	145	97.3	110	75.9*	94	85.7**	338	3.6*
CS2BTms	142	136	95.7	91	66.9	63	69.2	205	3.3
CS2DTms	165	165	100.0	79	47.9	32	40.5	96	3.0
CS3ATms	112	110	98.2	68	61.8	24	35.3*	77	3.2
CS3BTms	175	171	97.7	73	42.7*	22	30.4*	62	2.8
CS4ATms	148	147	99.3	96	65.3	45	46.9	162	3.6*
CS4BTms	129	127	98.4	105	82.7*	96	91.4**	394	4.1***
CS4DTms	147	147	100.0	121	82.3**	97	80.0*	194	2.0
CS5ATms	113	101	89.4	87	86.1**	66	75.9*	337	5.1***
CS5BTms	132	132	100.0	68	51.5	33	48.5	59	1.8*
CS5DTms	122	115	94.2	66	57.4	30	45.4	48	1.6*
CS6ATms	144	144	100.0	92	63.9	55	59.8	104	1.9*
CS6BTms	200	195	97.5	76	39.0*	8	10.5***	34	4.3***
CS6DTms	167	162	97.0	87	53.7	53	60.9	154	2.9
CS7ATms	169	169	100.0	68	40.2*	21	30.8*	59	2.8
CS7BTms	106	105	99.1	66	62.8	24	36.4	60	2.5
CS7DTms	147	146	99.3	92	63.0	48	52.7	125	2.6

Significant at *P = 0.05, **P = 0.01 and ***P = 0.001

propagation) as compared to the controls (parental cultivars) was observed for many substitution lines. The data indicated the crucial role of the genotype in later phases of the *in vitro* response and are in agreement with the observations of other authors (Henry *et al*. 1994b). These data suggest that the different phases are under the control of different genes or gene combinations. As was mentioned above, both parental cultivars used showed a similar *in vitro* response. Therefore, no considerable difference in the response to *in vitro* cultivation of the substitution lines was expected. However, the data showed that substitutions of certain chromosomes were accompanied by significant variation in the behaviour of the immature embryos during *in vitro* cultivation. These data indicated that the parental cultivars were not completely identical as to the alleles of the genes controlling TCR, but the differences were expressed in a different genetic background. Significant differences were established in the number of morphogenic calli and subsequently recovered regenerants. Much higher percentage of morphogenic calli and more regenerants were obtained in lines 2A, 5A, 4B and 4D. Chromosomes 3A, 1B, 3B, 6B and 1D affected the above-mentioned characteristics negatively. Chromosomes 2A, 4A, 5A, 4B and 6B exerted a positive effect on

the coefficient of propagation, with chromosomes 5A, 4B and 6B having the strongest one. Chromosomes 6A, 5B and 5D had an almost equally strong negative effect. Our data differ from the results of Kaleikau *et al*. (1989) according to which the 4B chromosome does not participate in somatic *in vitro* morphogenesis as well as from those of Felsenburg *et al*. (1987) according to which the 4B chromosome has a lesser effect upon the *in vitro* response compared to other chromosomes of Ch. Spring. Our results are in agreement with those of Mathias and Fukui (1986) and Higgins and Mathias (1987) indicating the strong influence of chromosome 4B.

In this study we determined the influence of 5A, 1B and 4D chromosomes and confirmed the effect of 2A, 3A, 3B, 4B, 6B and 1D chromosomes determined by other authors (Mathias & Fukui 1986; Higgins & Mathias 1987; Felsenburg *et al*. 1987; Henry *et al*. 1994a, b; Ben Amer *et al*. 1995, 1997). As was already pointed out, our results indicate that in spite of the fact that the two cultivars are similar in their *in vitro* response they possess different alleles for the genes modifying *in vitro* morphogenesis, or have differently linked genes on certain chromosomes. The difference in the allele status of certain genes in both cultivars is possibly expressed in the alien genetic background as a result of gene interaction (Salman 1986). The increased regeneration ability resulting from substitution of certain chromosomes from Timstein into Ch. Spring is probably due to different gene combinations and interactions. The negative effect of some Timstein chromosomes is probably caused by the different gene interactions, but in the opposite direction.

Cialacu and Săulescu (1998) suggested an association between morphogenic ability and the genes controlling β-amylase synthesis. According to Forsythe & Koebner (1992) the genes controlling β-amylase synthesis are located on chromosomal arms $5A^L$, $4B^L$ and $4D^L$. It is possible that the effect of chromosomes 5A, 4B and 4D on somatic morphogenesis might be explained by allele differences of the genes encoding the β-amylase isozymes.

The effect of the 1B chromosome substitution might be due to a difference in the allele status of the Gli 1B gene, encoding some gliadin fractions (Cialacu & Săulescu 1998), or to the genes tightly linked to the Gli-1B locus. As it is known, the marker gene for 1B Gli fractions is located on chromosome 1B and a strong effect of the gene Gli 1B on TCR of wheat was reported (Cialacu & Săulescu 1998). These suggestions need further investigations.

In conclusion, we determined the influence of the 5A, 1B and 4D chromosomes on somatic *in vitro* morphogenesis and confirmed the effect of the 2A, 3A, 4B, 6B and 1D chromosomes, reported by other authors. Besides, we found out that the intervarietal substitution might result in direct changes in the callus regeneration ability of substitution lines, in spite of the similarity of the two parental cultivars. This fact probably reflects the allele variation in a given locus on these chromosomes and/or background effect. Chromosome substitutions may have a different compensatory effect depending on the similarity of the corresponding chromosomes in strength and direction of gene action and also on the possibilities of their expression in an alien genetic background.

Further investigations, mainly molecular genetic analyses, will allow more detailed characterization of genes controlling somatic morphogenesis in wheat.

References

Ben Amer I.M., Worland A.J., Börner A. (1995): Chromosomal location of genes affecting tissue culture response in wheat. Plant Breeding, **114**: 84–85.

Ben Amer I.M., Korzum V., Worland A.J., Börner A. (1997): Genetic mapping of QTL controlling tissue culture response on chromosome 2B of wheat (*T. aestivum)* in relation to major genes and RFLP markers. Theoretical and Applied Genetics, **94**: 1047–1052.

Cialacu M., Săulescu N.N. (1998): Genetic analysis of somatic embryogenesis in winter wheat (*T. aestivum* L.). Romanian Agricultural Research, 9–10: 5–9.

Felsenburg T., Feldman M., Galun E. (1987): Aneuploid and alloplasmic lines as tools for the study of nuclear and cytoplasm control of culture ability and regeneration of scutellar calli from common wheat. Theoretical and Applied Genetics, **74**: 802–810.

Forsythe S.S., Koebner R.M.D. (1992): Wheat endosperm high molecular weight albumins and β-amylases: genetic and electrophoretic evidence of their identity. Journal of Cereal Science, **15**: 137–141.

Galiba G., Kovacs G., Sutka J. (1986): Substitution analysis of plant regeneration from callus culture in wheat. Plant Breeding, **97**: 261–263.

Henry Y., Vain D., De Buyser J. (1994a): Genetic analysis of *in vitro* plant tissue culture responses and regeneration capacities. Euphytica, 1–2: 45–58.

Henry Y., Marcotte J.L., De Buyser J. (1994b): Chromosomal location of genes controlling short-term and long-term somatic embryogenesis in wheat revealed by immature embryo culture of aneuploid lines. Theoretical and Applied Genetics, **89**: 344–350.

Higgins P., Mathias R.J. (1987): The effect of the 4B chromosomes of hexaploid wheat on the growth and regeneration of callus cultures. Theoretical and Applied Genetics, **74**: 439–444.

Kaleikau E.K., Sears R.G., Gill B.S. (1989): Monosomic analysis of tissue culture response in wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics, **78**: 625–632.

Mathias R.I., Fukui K. (1986): The effect of specific chromosome and cytoplasmic substitution on the tissue culture response of wheat (*T. aestivum*) callus. Theoretical and Applied Genetics, **71**: 797–800.

McIntosh R.A., Hart G.E., Gale M.D. (1991): Catalogue of gene symbols: 1991 supplement. Wheat Newsletter, **37**: 200–216.

Murashige T., Skoog F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology of Plant, **15**: 473–479.

Salman R. (1986): Genetic control of tissue culture response in winter wheat, *T. aestivum* (L.). [PhD Thesis.] Kansas State University.

Tyankova N.D., Zagorska N.A. (2001): Genetic control of *in vitro* response in wheat (*Triticum aestivum* L.). *In vitro* Cellular and Developmental Biology-Plant, **37**: 524–530.

Received for publication January 11, 2005
Accepted after corrections December 21, 2005

Corresponding author:

Dr. Nedyalka Dineva Tyankova, Institute of Genetics "Acad. D. Kostoff", Bulgarian Academy of Sciences, Sofia 1113, Bulgaria
e-mail: nellytyan@yahoo.com