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Genetic mapping of QTL controlling tissue-culture response on chromosome 2B of wheat (*Triticum aestivum* L.) in relation to major genes and RFLP markers

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Abstract Three quantitative trait loci (QTL) for tissue-culture response (*Tcr*) were mapped on chromosome 2B of hexaploid wheat (*Triticum aestivum* L.) using single-chromosome recombinant lines. *Tcr-B1* and *Tcr-B2*, affecting both green spots initiation and shoot regeneration, were mapped in relation to RFLP markers in the centromere region and on the short arm of chromosome 2B, linked to the photoperiod-response gene *Ppd2*. A third QTL (*Tcr-B3*), influencing regeneration only, was closely related to the disease resistance locus *Yr7*/*Sr9g* on the long arm of chromosome 2B. The homoeologous relationships to the tissue-culture response loci *Qsr*, *Qcg* and *Shd* of barley are discussed. A possible influence of the earliness *per se* genes of wheat and barley is suggested.

Key words Tissue-culture response · Wheat · Genetic mapping · RFLP · QTL

Introduction

The genetical control of tissue-culture response (TCR) in hexaploid wheat (*Triticum aestivum* L.) has been described in several reports including those of Shimada (1978), Sears and Deckard (1982), Maddock et al. (1983) and Lazar et al. (1983). Different chromosomes and chromosome arms have been identified as being correlated to different degrees with TCR traits (Galiba et al. 1986; Felsenburg et al. 1987; Kaleikau et al. 1989a; Henry et al. 1994), suggesting that differentiation and regeneration is polygenicly controlled.

Genes affecting the whole plant phenotype located on homoeologous group-2 and -4 chromosomes were suspected of promoting TCR by disturbing the hormone metabolism in callus cells (Mathias and Fukui 1986; Mathias and Atkinson 1988; Kaleikau et al. 1989a). Ben Amer et al. (1992a, 1996) suggested, however, that such genes might not be directly involved in TCR. Strong indications that chromosome 2B carries gene(s) having major effects on TCR were shown by Felsenburg et al. (1987), Kaleikau et al. (1989b), Henry et al. (1994) and Ben Amer et al. (1995).

The resolution of quantitatively inherited traits into their single-gene components via linkage with restriction fragment length polymorphism (RFLP) markers has recently become possible in wheat (Galiba et al. 1995; Worland 1996) as a result of the construction of genetic linkage maps containing large number of markers for chromosomes, including those of homoeologous group 2 (Devos et al. 1993). The identification of RFLP probes linked to genes promoting TCR will allow such genes to be located accurately and will enable their utilisation more efficiently in breeding programmes.

While in barley genes controlling tissue-culture traits have already been mapped on several chromosomes (Komatsuda et al. 1993, 1995; Mano et al. 1996), no such genes have so far been mapped in wheat. The present study was designed to identify loci associated with somatic tissue-culture ability on chromosome 2B in wheat. The approach involves the evaluation of two series of single-chromosome recombinant lines for chromosome 2B for tissue-culture response and a search for associations with segregating major genes and RFLP markers.

Materials and methods

Plant materials

In the first experiment, 81 single-chromosome recombinant lines (including six duplicates) resulting from a cross between wheat

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variety 'Chinese Spring' (CS) and a single-chromosome substitution line, 'CS/Marquis[1] 2B', in which the 2B chromosome of 'CS' had been replaced by its homologue from 'Marquis[1]'[1] were studied. Procedures for the development of homozygous recombinant lines were as described by Law (1966). The extracted lines are homozygous recombinant for the chromosome of interest (2B) in the genetic background of CS. The material was chosen because 'CS/Marquis[1] 2B' has been shown recently to have an increased regeneration efficiency compared to 'CS' (Ben Amer et al. 1995).

The single-chromosome recombinant lines had been classified earlier (Scarth and Law 1983) for marker genes for photoperiod response (*Ppd2*), yellow rust (*P. striiformis*) resistance (*Yr7*) and stem rust (*P. graminis*) resistance (*Sr9g* and *Sr16*). The genes *Yr7* and *Sr9g* were found to be co-segregating. A further classification of the lines was carried out by Leckie et al. (1988) who mapped a gene for resistance to the wild oats herbicide difenzoquat (*Dfq1*).

In the second experiment an RFLP analysis was initiated using the wheat variety 'Cappelle-Desprez' (Cap), which carries the recessive allele for photoperiod sensitivity *ppd2*, a single-chromosome substitution line in which the 2B chromosome of 'Cap' had been replaced by its homologue from 'CS' carrying the dominant allele for photoperiod insensitivity *Ppd2* and 61 single-chromosome recombinant lines derived from a cross between 'Cap' and the substitution line 'Cap/CS 2B'.

Tissue-culture response

From each genotype, two main spikes were harvested 14 to 16 days after anthesis from four to six plants grown in the greenhouse. Fifteen immature embryos per spike were cultured (5 per petri dish), as described by Sears and Deckard (1982), on initiation medium (I-Med.) containing 2.0 mg/l 2,4-dichlorophenoxyacitic acid (2,4-D). Calli were transferred twice at 4-week intervals onto maintenance (M-Med.) and regeneration (R-Med.) media containing 0.5 and 0.1 mg/l 2,4-D, respectively. The handling of the explants and the growth conditions of the cultures were as described previously (Ben Amer et al. 1992b).

RFLP analysis

Total DNA of each 'Cap/CS 2B' single-chromosome recombinant line as well as of both parental lines were extracted from green leaves of 5- to 6-week-old plants following McCouch et al. (1988). All other RFLP techniques were performed as described by Devos et al. (1992), except that denaturation of the labelled probe was done by the addition of 1/10 volume 3 *M* NaOH.

Out of 32 selected cDNA and genomic DNA probes known to be located on wheat chromosome 2B and kindly supplied by M. D. Gale, John Innes Centre Norwich, UK, 14 gave polymorphisms between the parents and were used to analyse the 62 'Cap/CS 2B' single-chromosome recombinant lines. Multipoint linkage values in centiMorgans (cM) (Kosambi 1944) were calculated using the programme MAPMAKER 2.0 supplied by E. S. Lander, Whitehead Institute of Biomedical Research, Cambridge/Mass., USA.

Statistical analysis

The first experiment was arranged in a randomised complete block design and the second one in a randomised design. The TCR response of the genotypes studied was scored as the percentage of calli with green spots (differentiation) at the end of the 4th week on M-Med. and the percentage of calli producing plantlets (regeneration) at the end of the 4th week on R-Med. The percentages for both traits were scored for each petri dish separately, and afterwards the mean percentages were calculated for each recombinant line. The means of the two parents were used to classify the recombinant lines as responsive or non-responsive.

Analysis of variance (ANOVAs) for the arcsin $\sqrt{}$% transformed means were used to detect differences between recombinant lines and to partition the variations into allelic effects of the chromosome 2B marker genes on the TCR traits for 'CS/Marquis[1] 2B' recombinant lines. ANOVAs were also used to detect associations between RFLP alleles and tissue-culture traits for the 'Cap/CS 2B' lines by comparing the variation between the two allele classes at each marker locus with the variation between lines within classes (Laurie et al. 1994). Correlation coefficients between the TCR traits green spots initiation and regeneration were calculated for each experiment.

Results

While the parental line 'CS' showed low frequencies of differentiation and regeneration (34.5% and 26.7%, respectively), the substitution of chromosome 2B of 'Marquis[1]' into 'CS' significantly increased the percentage of both traits up to 53.9% and 46.0%, respectively. On the other hand, the frequencies of differentiation and regeneration from 'Cap' were 60.0% and 46.7%, respectively, and these frequencies were significantly reduced to 34.7% and 23.4%, respectively, upon the substitution of chromosome 2B of 'CS' into 'Cap' (Fig. 1).

Correlation coefficients between differentiation and regeneration for the 'CS/Marquis[1] 2B' and the 'Cap/CS 2B' recombinants were $r = 0.841$*** and $r = 0.944$***, respectively, suggesting that the two traits have at least some gene(s) in common.

CS/Marquis[1] 2B recombinant lines

The frequency distributions for green spots initiation (Fig. 1A) and regeneration (Fig. 1B) gave no clear-cut 1:1 segregation, although there was some indication for a discontinuous distribution, suggesting the presence of major genes. The statistical analysis (Table 1) showed a highly significant 'overall line' variation between the recombinant lines for differentiation and regeneration, indicating that the two traits are under genetical control. The few duplicate lines included showed no significant differences for both TCR traits.

For the differentiation response, the analysis for each marker gene showed that only *Ppd2* vs. *ppd2* was significant ($P = 0.05$–0.01). The removal of the variation due to each of the other marker genes from 'between-line' variation left a residual which was highly significant (Table 2). These results indicated that the genes *Yr7*/*Sr9g*, *Sr16* and *Dfq1* were not involved in the differentiation. For the *Ppd2* gene, however, the

[1] The 'Marquis' selection used in developing the 'Chinese Spring/Marquis' substitution lines was not a true 'Marquis'. It was probably derived from a cross with var 'Thatcher' (Sheen and Snyder 1964; Scarth 1981; McIntosh, personal communication)

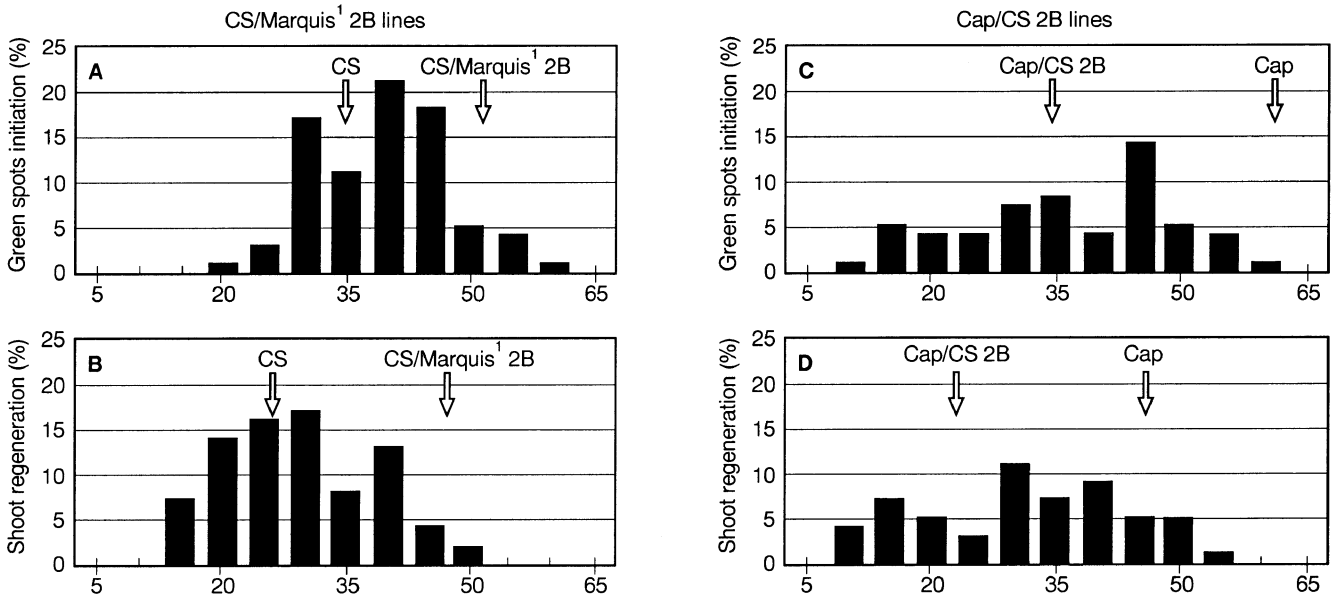

Fig. 1A–D Segregation patterns for the traits green spots initiation (**A** and **C**) and shoot regeneration (**B** and **D**) of the 'CS/Marquis[1] 2B' (**A** and **B**) and 'Cap/CS 2B' (**C** and **D**) single-chromosome recombinant lines. The parental means are marked by the *arrows*

Table 1 ANOVA for the TCR traits green spots initiation and shoot regeneration amongst the 'CS/Marquis[1] 2B' recombinant lines (arcsin $\sqrt{\%}$ transformation, $*P = 0.05–0.01$, $**P = 0.01–0.001$, $***P < 0.001$)

Source of variation	*df*	MS	
		Green spot initiation (%)	Shoot regeneration (%)
Block	3	329.800*	191.800
Overall line	86	260.444**	269.651**
Duplicates	6	82.884	21.652
Between line	80	278.951**	317.276**
Responsive vs. non-responsive	1	13280.386***	17371.640***
Residual	79	114.376	102.081
Error	248	113.808	93.436

removal of the *Ppd2* vs. *ppd2* comparison from 'between-line' variation left also a residual which was still highly significant ($P = 0.01–0.001\%$). The results suggested the involvement of at least two genes affecting differentiation, *Ppd2* or a closely linked gene and (an)other genetical factor(s), not linked to any of the marker genes.

The data obtained on regeneration response (Table 2) indicated that the marker genes *Ppd2*, *Yr7/Sr9g* and *Dfq1* were strongly associated with this trait. Because *Yr7/Sr9g* and *Dfq1* are closely linked (Leckie et al. 1988) we concluded that at least two genes of the 'Marquis[1]' 2B chromosome were responsible for an increased regeneration frequency. One was found to be located again in the *Ppd2* gene region on chromosome 2BS, and the other is believed to be present near the loci of *Yr7/Sr9g* and *Dfq1* on the long arm of 2B.

'Cap/CS 2B' recombinant lines

In confirmation of the results of the first experiment the frequency distributions for green spots initiation and regeneration were again found to be discontinuous (Fig. 1C, D) with, however, no clear cut 1 : 1 segregation.

The ANOVAs for the differentiation response (Fig. 2) showed a strong association with RFLP marker *Xpsr126* ($P < 0.001$) which mapped in the centromere region on the short arm of chromosome 2B about 13 cM proximal to the photoperiod response gene *Ppd2* (Worland et al. 1997). Furthermore, the analysis showed that a second locus, *Xpsr666*, located 5.5 cM distal to *Ppd2* on chromosome 2BS was also associated ($P = 0.05–0.01$) with callus differentiation.

For plantlet regeneration again the markers *Xpsr126* ($P < 0.001$) and *Xpsr666* ($P = 0.01–0.001$) showed a strong association, indicating that these loci were involved in both differentiation and regeneration. The loci of the two markers explained 25% of the genetic variance for plant regeneration. The *Ppd2* gene and other RFLP markers located between *Xpsr126* and *Xpsr666* (Fig. 2) were associated slightly with regeneration ($P = 0.05–0.01$), which may reflect a linkage relationship between the two loci for tissue-culture response. Unfortunately, only three RFLP markers were mapped on 2BL for the 'Cap/CS 2B' recombinant lines. *Xpsr102* was mapped close to the centromere on the long arm of chromosome 2B with a distance of 1.7 cM to the co-segregating markers *Xpsr126*, *Xpsr380* and *Xpsr146*, belonging to 2BS. The markers

Table 2 ANOVA for the TCR traits green spots initiation and shoot regeneration amongst the 'CS/Marquis[1] 2B' recombinant lines using the partitioning of the lines into classes according to the major genes *Ppd2*, *Yr7/Sr9g*, *Dfg1* and *Sr16*. Only the lines with complete classification data (68) were used (arcsin $\sqrt{}$% transformation; **P* = 0.05–0.01, ***P* = 0.01–0.001, ****P* < 0.001)

Source of variation	*df*	MS	
		Green spot initiation (%)	Shoot regeneration (%)
Between line	68	255.726**	303.847**
Ppd2 vs. *ppd2*	1	450.400*	2418.364***
Residual	67	252.857**	272.287**
Yr7/Sr9g vs. *yr7/sr9g*	1	110.525	783.290**
Residual	67	257.930**	296.691**
Dfg1 vs. *dfg1*	1	73.449	786.080**
Residual	67	258.483**	296.649**
Sr16 vs. *sr16*	1	62.221	105.241
Residual	67	258.651**	306.811**
Error	194	114.812	87.396

Xpsr934 and *Xpsr609* were mapped 16.7 cM and 26.5 cM distal to *Xpsr102*, respectively (Worland et al. 1997). Only *Xpsr102* was found to be weakly involved in the regeneration response ($P = 0.05$–0.01).

Discussion

Studying 'Cap/Mara 2D' single-chromosome recombinant lines differing in alleles at the *Ppd1* and *Rht8* loci, Ben Amer et al. (1992b) demonstrated that the lines carrying the photoperiod-sensitive allele *ppd1* promoted regeneration. On the other hand, the substitution of chromosome 2B from 'Marquis[1]' and 'Cheyenne', both carrying the photoperiod-sensitive allele *ppd2*, into 'CS' background led to a significant increase and decrease, respectively, in plant regeneration (Ben Amer et al. 1996). These results suggest that a gene(s) linked to the *Ppd* might be involved in tissue-culture response.

By combining the traditional major gene segregation method with the more advanced RFLP analysis method in the present investigation we were able to detect and map quantitative trait loci (QTL) on chromosome 2B controlling somatic tissue-culture traits in wheat. The results reported here showed two QTL controlling differentiation (green spots initiation), one locus '*Tcr-B1*' (*Tcr* = Tissue culture response) with a stronger effect located in the centromere region and another locus '*Tcr-B2*' with a moderate effect on the short arm of chromosome 2B, distal to *Ppd2* and closely linked by approximately 5 cM (Fig. 3). Both QTL were also detected for the character callus regeneration, thereby confirming the high correlation observed between these two traits in both this ($r \geqslant 0.841$***) and previous studies (Ben Amer et al. 1995, 1996). Henry et al. (1994), and Felsenburg et al. (1987) reported the involvement of 2BS in differentiation and regeneration while, conversely, Kaleikau et al. (1989b) reported the absence of an effect of 2BS on both traits. The results presented here confirm our earlier indication of the presence of *Tcr* genes in the *Ppd* gene region (Ben Amer et al. 1992b, 1996).

Henry et al. (1994) classified calli of green spots into two classes, one with and one without somatic embryos. They suggested that a gene controlling the production of green spots lacking somatic embryos is located on 2AS. It is possible that this locus may be homoeoallelic to '*Tcr-B2*' on 2BS observed in this study.

A third QTL, '*Tcr-B3*', influencing regeneration but not differentiation was detected in 'CS/Marquis[1] 2B' recombinant lines linked to the disease-resistance locus *Yr7/Sr9g* on the long arm of chromosome 2B, about 20 cM distal from the centromere (Sears and Loegering

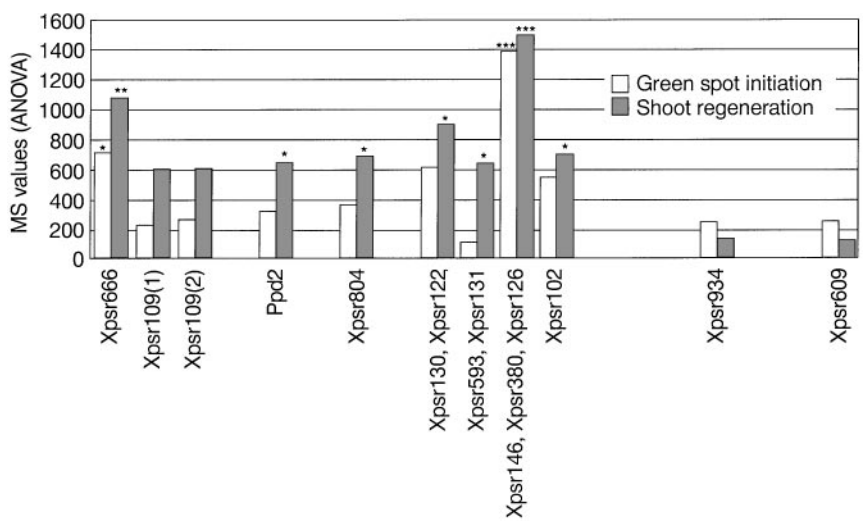

Fig. 2 Mean square values (ANOVA) and levels of significance for the TCR traits green spots initiation and shoot regeneration amongst the 'Cap/CS 2B' recombinant lines using the partitioning of the lines into classes according to RFLP markers (**P* = 0.05–0.01, ***P* = 0.01–0.001, ****P* < 0.001)

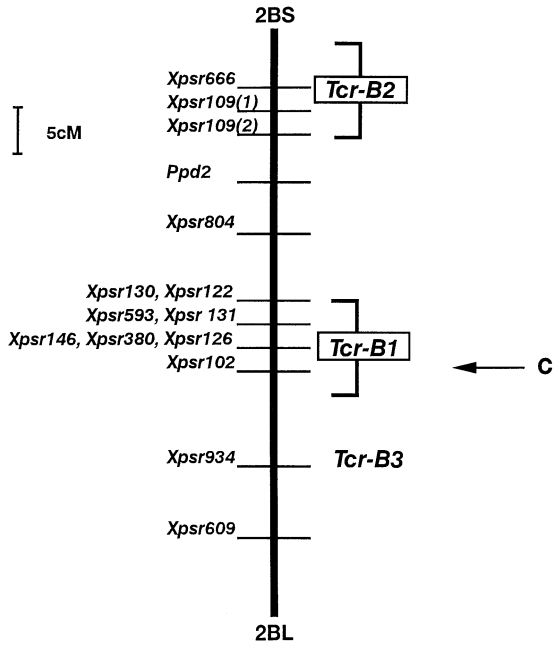

Fig. 3 Genetic map of chromosome 2B showing the location of the QTL *Tcr-B1* and *Tcr-B2* (*c* centromere). The probable location of *Tcr-B3* is included

1968). This locus was not detected when the 'Cap/CS 2B' recombinant lines were analysed, suggesting that a similar allele is present in both these varieties. The loci *Xpsr934* and *Xpsr609*, mapped about 17 cM and 27 cM, respectively, distal to the centromere on 2BL by Worland et al. (1997), were not associated with any of the tissue-culture response traits. In earlier homoeologous group-2 RFLP mapping studies (Devos et al. 1993) however, the distance of both markers to the centromere was more than 50 cM. Therefore, further studies are needed to determine the exact location of *Tcr-B3*. Nevertheless, this result is in agreement with the results reported by Henry et al. (1994), indicating that 2BL carries a gene promoting regeneration. On the other hand, Kaleikau et al. (1989b) suggested that 2BL carries a 'regulator' gene controlling the expression of TCR traits.

In barley, chromosome 2H was shown to influence shoot regeneration. Komatsuda et al. (1993, 1995) identified the QTL *Shd1* (*Shoot differentiation*). This locus was mapped by RFLPs in the chromosomal region containing the *v* gene, which determines the 2-row/6-row ear type on 2HL, and it may be homoeoallelic to *Tcr-B3* of wheat. A further locus, *Qsr1* (*Quantitative trait locus for shoot regeneration*), was mapped recently by Mano et al. (1997) in the centromere region of chromosome 2H, probably homoeoallelic to *Tcr-B1*, whereas another QTL controlling callus growth rate, *Qcg1* (*Quantitative trait locus for callus growth*), was located again on the long arm of chromosome 2H.

Interestingly, genes modifying ear emergence time independently of environmental stimuli (vernalisation, photoperiod), 'earliness *per se*' genes (*eps*) were recently mapped in the centromere regions of chromosome 2B of wheat (Worland 1996) and 2H of barley (Laurie et al. 1994), respectively. These genes act through the determination of the number and/or the rate of primordia initiation (Worland et al. 1997). In similar way, the *eps* genes could influence the number and/or the rate of the differentiated cells in tissue culture as secondary pleiotropic effects. Earliness *per se* genes of wheat have been located on chromosomes 2B, 3A, 4B, 4D, 6B, 6D and 7B (Worland 1996). Most of these chromosomes have also been reported to influence tissue-culture response (Mathias and Fukui 1986; Galiba et al. 1986; Felsenburg et al. 1987; Henry et al. 1994; Ben Amer et al. 1996). In barley a further QTL for tissue-culture response – *Qsr3* – was located by Mano et al. (1996) in the same region of chromosome 6HL where the *eps6L.2* locus was mapped by Laurie et al. (1995). All these findings suggest that the earliness *per se* genes may influence the tissue-culture performance of the Triticeae.

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