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An N-band marker for gene *Lr18* for resistance to leaf rust in wheat

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Abstract The leaf rust resistance gene, *Lr18*, of common wheat cultivars has been derived from *Triticum timopheevi* and is located on chromosome arm 5BL. Chromosome banding (N-banding) analyses revealed that in the wheat cultivars carrying *Lr18* that were examined, which had been bred in 6 different countries, chromosome arm 5BL possessed a specific terminal band not carried by their susceptible parental cultivars. It was suggested that this terminal N-band was introduced from *T. timopheevi* together with *Lr18*. N-banding analysis of a *T. timopheevi* strain showed that one of two *timopheevi* chromosomes had provided Japanese wheat lines containing *Lr18* with the terminal band.

Key words Leaf rust resistance · *Lr18* · N-banding · Wheat chromosome arm 5BL

Introduction

Several common wheat (*Triticum aestivum* L.) cultivars possess alien genes that have been derived from related species (Sharma and Gill 1983). Some genes for resistance to leaf rust disease (*Lr* gene) have also been introduced from alien species, e.g., *Lr9* from *Aegilops umbelullata* and *Lr26* from *Secale cereale* (McIntosh 1988). The gene *Lr18* which is located on the long arm of wheat chromosome 5B (5BL) has been introduced from *T. timopheevi* into common wheat cultivars in different wheat breeding programs (McIntosh 1983).

The differential staining techniques of C- and N-banding have proven to be effective in identifying mitotic chromosomes of wheat-alien translocations (Gill and Kimber 1977; Friebe et al. 1991) as well as those of wheat and its relatives (Gill and Kimber 1974; Gerlach 1977). The transfer of *Lr18* from the *T. timopheevi* genome to wheat chromosome arm 5BL may have generated an altered banding pattern of the arm that is detectable by these banding techniques. This report describes how a specific terminal N-band was found on chromosome arm 5BL of wheat cultivars carrying *Lr18*.

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Materials and methods

Wheat materials

Ten Japanese wheat lines resistant to leaf rust were used. Their cross combinations are shown in Table 1. These resistant lines were bred at the Tohoku National Agricultural Experiment Station. The *Lr18* resistance gene of wheat line 'Sabikei 12' (McIntosh 1983) was introduced from *Triticum timopheevi* Zhuk. (genome constitution AAGG, $2n = 28$) accession no. 2 through another resistant wheat line, 'FTF'. The other 9 'Sabikei' lines were produced using 'FTF' as the source of leaf rust resistance, and it remained unknown whether these lines carried *Lr18* or not. The reactions of some 'Sabikei' lines to Japanese races of leaf rust fungus (*Puccinia recondita* Rob. ex. Desm.) have been reported by Mukade et al. (1969).

Eleven international wheat cultivars or lines carrying *Lr18* (McIntosh 1983) were also examined: 'Timvera' (cross combination; 'Steinwedel'/*T. timopheevi*) and 'Timvera Derivative' ('Gabo'/'Timvera') from Australia; 'RL 6009' ('South Africa 43'/7* 'Thatcher') and 'RL 6090' ('Sabikei 12'/6 *'Thatcher') from Canada; 'Red Egyptian PI 17016–2c' from Egypt; 'South Africa 43' from South Africa; 'WYR 50847', 'WYR 50853', 'WYR 50854', 'WYR 50855', and 'WYR 50858' from Russia. These materials were provided by Drs. R. A. McIntosh (University of Sydney, Australia), P. L. Dyck (Agriculture Canada Research Station, Canada), and R. S. Pandeya (Plant Gene Resources of Canada, Canada). Three parents not possessing *Lr18*, i.e., cvs. 'Steinwedel', 'Gabo', and 'Thatcher', were also examined.

Chromosome banding technique

Mitotic chromosomes of root-tip cells were studied by the N-banding technique (Endo and Gill 1984), which can identify wheat chromosome 5B. The roots examined were collected from at least 5 seedlings for each cultivar.

Table 1 Pedigrees of wheat lines 'Sabikei' and 'FTF' examined by N-banding

Line	Cross Combination
Sabikei 12	Tohoku 118/FTF//Tohoku 118
Sabikei 11	Tohoku 118/FTF//Tohoku 118
Sabikei 1	Aobakomugi/FTF
Sabikei 3	Furutsumasari/FTF//Furutsumasari
Sabikei 4	Furutsumasari/FTF//Furutsumasari
Sabikei 5	Nanbukomugi/FTF//Nanbukomugi
Sabikei 16	Shimofusakomugi/FTF//Shimofusakomugi
Sabikei 17	Miyaginokomugi/FTF//Miyaginokomugi
Sabikei 18	Kokeshikomugi/FTF//Kokeshikomugi
Sabikei 19	Furutsumasari/FTF//Tohoku 118
FTF	Fultz no. 1/*T. timopheevi*//Fultz no. 1

Table 2 Allelism test on the reaction to leaf rust among 10 'Sabikei' lines (*Res* resistant reaction · *Int* intermediate reaction · *Sus* Susceptible reaction)

Cross combination and wheat lines	Number of plants		
	Res	Int	Sus
F_2 generation			
Sabikei 12/Sabikei 1	181	0	0
Sabikei 12/Sabikei 3	144	5	0
Sabikei 12/Sabikei 4	171	6	0
Sabikei 12/Sabikei 5	196	0	0
Sabikei 12/Sabikei 11	185	0	0
Sabikei 12/Sabikei 16	191	1	0
Sabikei 12/Sabikei 17	189	4	0
Sabikei 12/Sabikei 18	176	1	0
Sabikei 12/Sabikei 19	185	0	0
Parental lines			
Sabikei 1	11	0	0
Sabikei 3	9	1	0
Sabikei 4	13	0	0
Sabikei 5	27	0	0
Sabikei 11	37	0	0
Sabikei 12	21	0	0
Sabikei 16	28	2	0
Sabikei 17	37	0	0
Sabikei 18	39	0	0
Sabikei 19	40	0	0
Control lines			
FTF	24	0	0
Tohoku 118	0	0	18
Nanbukomugi	0	0	20

Inoculation test by leaf rust

Spores of leaf rust fungus collected from leaves of a susceptible wheat, cv 'Nanbukomugi', were used as inoculum that would contain several kinds of races. The inoculation test was conducted on 'Sabikei' lines to determine the presence/absence of resistance gene *Lr18*.

Spores were inoculated onto the seedlings at the two- to three-leaf stage. After inoculation, the seedlings were grown at about 15 °C as 'FTF', the source of *Lr18*, shows a resistance reaction to races 37B and 21B of leaf rust at 20 °C but a susceptible reaction at 27 °C (Kamio and Mukade 1969). The infection types were classified according to Yamada et al. (1960). In this study, infection types 0 (no visible infection: chlorotic or necrotic areas without uredia) and 1 (minute uredia surrounded by necrotic areas) were considered to be resistant; types 2 (small uredia with necrosis) and 3 (medium uredia surrounded by chlorosis) were intermediate; and type 4 (large uredia without chlorosis and necrosis) was susceptible.

Results and discussion

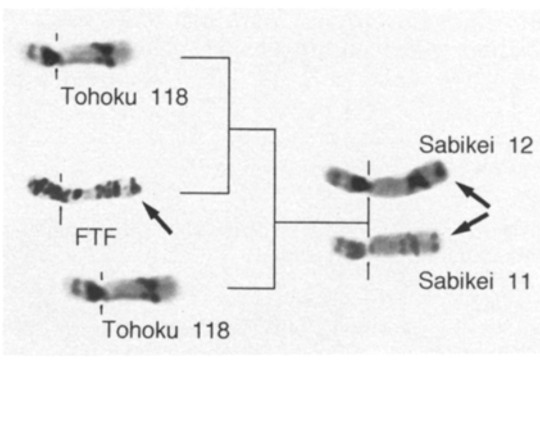
Presence of *Lr18* in leaf rust-resistant 'Sabikei' lines of wheat

Since 'FTF' was the resistance source for 9 'Sabikei' lines ('Sabikei 1', '3', '4', '5', '11', '16', '17', '18', and '19') as well as for 'Sabikei 12', which carries *Lr18* (McIntosh 1983), an allelism test was carried out for leaf rust resistance to confirm the presence of *Lr18* in these 9 lines. Cross combinations for the individual F_2 progenies, and results of the segregation are shown in Table 2. No typically susceptible plants segregated in the F_2 generation in any of the crosses, although a few plants showed an intermediate reaction. The inoculum may consist of several kinds of leaf rust races, thus causing the infrequent intermediate reaction to appear. Consequently, these findings were taken to indicate that *Lr18* had been repeatedly introduced into all 'Sabikei' lines and that the effective resistance of 'FTF' results from *Lr18*.

N-banding analysis of chromosome 5B in 'Sabikei' lines of wheat

In order to clarify whether or not chromosome arm 5BL possesses a specific banding pattern caused by the transfer of *Lr18*, the N-banding patterns of chromosome 5B in 'Sabikei 11', 'Sabikei 12', 'Tohoku 118', and 'FTF' were compared. The wheat chromosome arm 5BL in 'Sabikei 11' and '12' had a small terminal N-band, three small N-bands (appearing as two due to band fusion) clustering in the intercalary region, and a faint centromeric band (Fig. 1). The 5BL of 2 'Sabikei' lines and 'Tohoku 118' lacked the two centromeric bands that were found in 'FTF'. The intercalary bands of the 'Sabikei' lines seemed to be more similar to those of 'Tohoku 118' than to those of 'FTF', while the terminal N-band was present in the 'FTF' and 'Sabikei' lines but

Fig. 1 N-banded 5B chromosomes of 'Sabikei 11', 'Sabikei 12', and their ancestral lines. *Arrows* indicate the specific terminal band on chromosome arm 5BL carrying the resistance gene *Lr18*. The *vertical lines* show the position of the centromere: in each chromosome, the short arm is to the *left* and the long arm is to the *right*



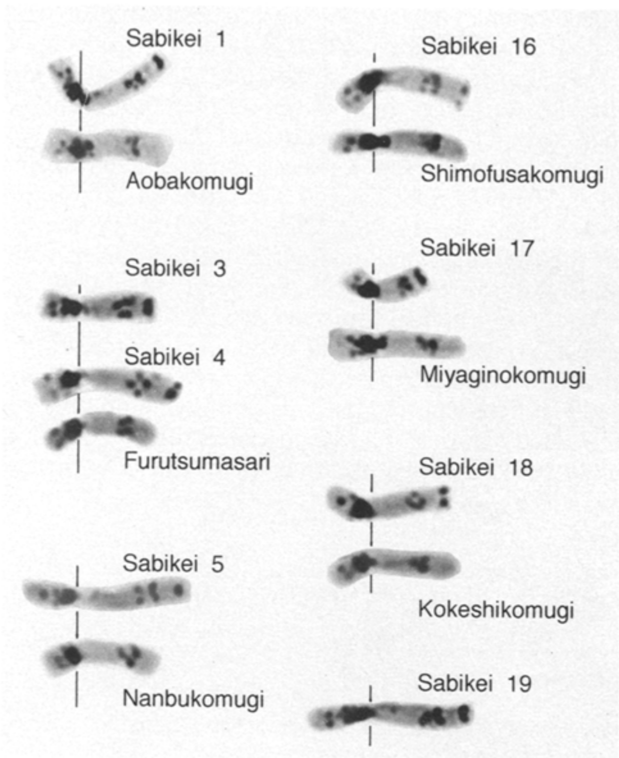


Fig. 2 N-banded 5B chromosomes of 8 'Sabikei' lines and their susceptible parents. All 'Sabikei' lines produced the terminal band on chromosome arm 5BL

not in 'Tohoku 118' (Fig. 1). Since McIntosh (1983) demonstrated that *Lr18* recombines freely to the centromere, this terminal band was assumed to be related to *Lr18*.

The other 8 'Sabikei' lines and their susceptible parents, i.e., 'Aobakomugi', 'Furutsumasari', 'Nanbukomugi', 'Shimofusakomugi', 'Miyaginokomugi', and 'Kokeshikomugi', were also subjected to N-banding (Fig. 2). The relationship between the 8 'Sabikei' lines and their parents was the same as that observed between 'Sabikei 11', 'Sabikei 12', and their parental lines. All 'Sabikei' lines had a terminal band on 5BL, but the susceptible parents did not. The intercalary and centromeric bands on the 5BL of 'Sabikei' lines were similar to those of the susceptible parents.

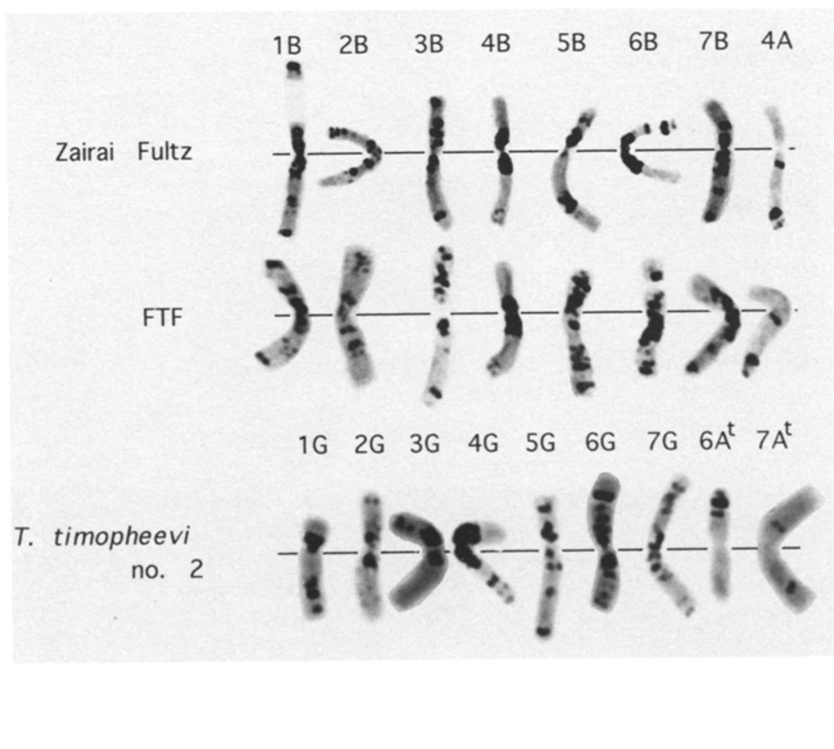
Since *Lr18* and the terminal band on chromosome arm 5BL coexisted in 'Sabikei' lines while their susceptible parents did not possess the terminal band, this band must originate from 'FTF' and be linked to *Lr18* in 10 'Sabikei' lines. Furthermore, the similarity of the intercalary bands of 'Sabikei' lines and susceptible cultivars indicates that the translocation point in the 5BL of the 'Sabikei' lines is situated between the intercalary and terminal bands.

The origin of the 5BL terminal band in *T. timopheevi* chromosomes

'FTF', the resistance donor to the 'Sabikei' lines, was bred from a cross, 'Fultz no. 1'/*T. timopheevi* accession no. 2//'Fultz no. 1' (Watanabe et al. 1959). In order to specify the origin of the terminal N-band on 5BL of 'FTF', *T. timopheevi* no. 2 and 'Zairai Fultz' were examined (Fig. 3). 'Fultz no. 1' could not be analyzed because it was not available in the wheat germ plasm collection. As 'Fultz no. 1' was bred by pure line separation from 'Zairai Fultz', the latter was examined in place of 'Fultz no. 1'.

'Zairai Fultz' did not possess a terminal N-band on 5BL. In *T. timopheevi* no. 2, N-bands appeared on 9 pairs of chromosomes, which were arranged in accordance with Badaeva et al. (1991). Seven pairs (1G–7G) of these had bands in or close to the terminal region on the

Fig. 3 N-banding patterns of chromosomes in wheat lines 'Zairai Fultz', 'FTF' and *T. timopheevi* no. 2



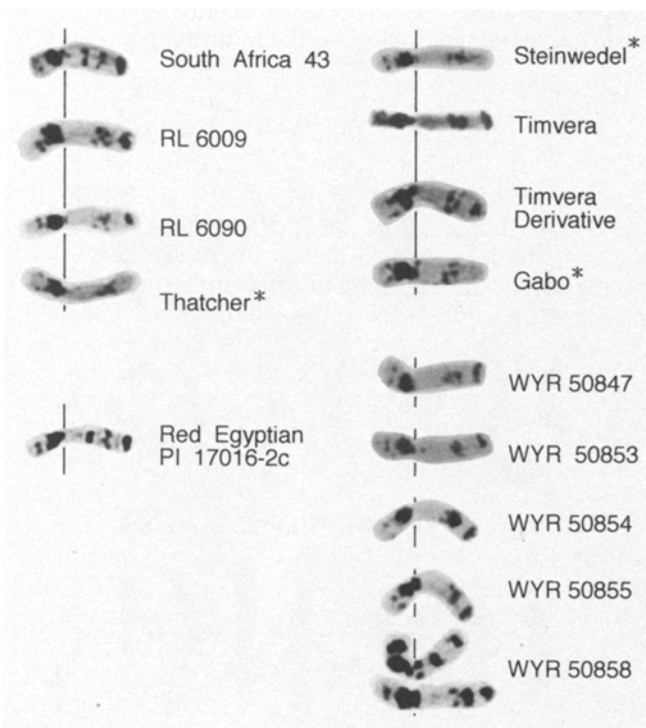
long or short arms. These results suggest that the terminal band had been derived from *T. timopheevi*. Compared to chromosome 5B in 'FTF', two *timopheevi* chromosomes, 1G and 5G, were considered as candidates for the donor of the terminal band to 'FTF'; both were submedian chromosomes having a terminal band on the long arm like chromosome 5B of 'FTF'. However, they differed from the latter in the following points. Chromosome 1G lacked two centromeric bands on the long arm and an intercalary band on the short arm, and the terminal band on the long arm was located a short distance from the end. Chromosome 5G had only one intercalary band on the long arm.

From these observations, the terminal N-band on wheat chromosome arm 5BL in the 'Sabikei' lines was suggested to have been derived from a *T. timopheevi* chromosome homoeologous to wheat chromosome 5B, through 'FTF'.

Chromosome 5B in foreign wheat cultivars and lines possessing *Lr18*

In 11 international wheat cultivars and lines carrying *Lr18* that were bred in 5 different countries, all of the 5BL arms had a terminal N-band (Fig. 4). One wheat line, 'WYR 50858', revealed two types of banding pattern of chromosome 5B in the homozygous or heterozygous condition, indicating that this line has not yet been fixed.

Fig. 4 N-banding patterns of 5B chromosomes in international wheat cultivars and lines possessing *Lr18* and in three of their parents (marked with $*$). All of the wheats carrying *Lr18* produced the terminal band on 5BL while the three parents did not



'Steinwedel' (a parent of 'Timvera'), 'Gabo' (a parent of 'Timvera Derivative'), and 'Thatcher' (the recurrent parent of both 'RL 6009' and 'RL 6090'), which do not carry *Lr18*, did not produce the terminal band on 5BL. Therefore, the terminal band of 'Timvera' must have been derived from *T. timopheevi*, and was then transmitted to 'Timvera Derivative'. Similarly, 2 near-isogenic lines of 'Thatcher', i.e., 'RL 6009' and 'RL 6090', have not lost the terminal band during recurrent crossing, which means that this terminal band is closely linked to *Lr18*.

The cytogenetical analyses described revealed that there is a specific terminal N-band on chromosome arm 5BL of wheat cultivars carrying *Lr18* that have been bred in different countries. This terminal band probably originated from a *T. timopheevi* chromosome in all cultivars and may be useful as a chromosomal marker for *Lr18*.

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