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Seedling lethality in wheat: a novel phenotype associated with a 2RS/2BL translocation chromosome

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Summary. When grown at normal temperatures, wheat plants disomic for a 2RS/2BL translocation chromosome substituting for chromosome 2B show seedling lethality. Morphological and physiological studies could not determine the cause of death. However, the seedling lethality can be partly to completely inactivated at higher temperatures and in stressed environments. The lethality can also be completely suppressed if the translocated chromosome is introduced into different wheat cultivars. These wheats must contain genes which suppress the lethal phenotype caused by disomy of 2RS/2BL.

Whilst the temperature effect indicates that the seedling lethality is related to the grass clump dwarf phenotype of wheat, our results show that the genes involved in seedling lethality, its suppression and inactivation, are not related to the *D* genes which cause grass clump dwarfing in wheat.

Key words: Wheat-rye-translocations – Seedling lethals – Grass clump dwarfs – In situ hybridization – Temperature sensitivity

Introduction

The modification of the hexaploid wheat genome (*Triticum aestivum* L. em Thell; $2n=6x=42$) by the substitution of alien chromosomes obtained from various of the diploid and tetraploid relatives of wheat is becoming a common procedure for the introduction of new agronomic characters into commercial wheats (Feldman and Sears 1981). If translocations between the wheat and alien chromosome are then induced – and these often occur when plants contain these chromosomes as univalents – these can be preferable to whole chromosome substitutions because the amount of alien chromatin is reduced and the chance of introducing undesirable characteristics is lessened. Conversely, translocations may also lead to the deletion of vital wheat genes or cause unfavourable interactions between wheat and alien gene products. A translocation with such an undesirable result is the 2RS/2BL chromosome recovered from a breeding programme designed to introduce chromosome 2R of rye (*Secale cereale* L. cv. 'Imperial') into commercial cultivars of wheat (May and Appels 1978, 1980). When this translocation replaces chromosome 2B entire, plants containing the disomic translocation show seedling lethality.

Our earlier interpretation of the biological effect of the 2RS/2BL chromosome was that the seedlings survive only until seed reserves expire. However, we have found that the seedling lethality is at least partially overcome by growing the plants at higher temperatures. As this response is similar to the response shown by grass clump dwarfs of wheat (Farrer 1898; McMillan 1937; Hermsen 1967) to increased temperatures (Canvin and Evans 1963; Canvin and McVetty 1976), it was necessary to determine whether the seedling lethality was an extreme variant of the grass clump dwarf phenotype. The 2RS/2BL translocation was therefore introduced into plants containing defined wheat dwarfing genes. Evaluation of subsequent progenies has shown that although the seedling lethality may be phenotypically related to the grass clump dwarf phenotype, it does not appear to be genetically related. Furthermore, the lethality can be overcome if 2RS/2BL is introduced into different genetic backgrounds.

Materials and methods

We have previously described the pedigrees of the wheat plants in which chromosomes 2R of rye, 2B of wheat, 2BS/2RL and 2RS/2BL were found (May and Appels 1978). In brief, 'Chinese Spring' 2R(2B) wheat (Sears 1968) was crossed with the wheat 'Tr535' (='Sun17F') and the F_1 plant was backcrossed to 'Timgalen'. The entire and translocated chromosomes were identified in subsequent generations using the in situ hybridization of molecular probes specific to either rye or wheat chromatin (May and Appels 1980).

Only plants disomic for the 2RS/2BL translocation (i.e. $20''+2RS/2BL''$) show seedling lethality. Plants with the karyotypes $20''+2B''$, $20''+2BS/2RL''$, $20''+2R''$, $20''+2R'+2BS/2RL'$ and $20''+2B'+2RS/2BL'$ differ little in vigour and vitality. To obtain new chromosomal combinations, the two heterozygotes were intercrossed and their progenies assayed by in situ hybridization before growing in the greenhouse. Plants containing three of the new combinations ($20''+2B'+2R'$, $20''+2B'+2BS/2RL'$ and $20''+2BS/2RL'+2RS/2BL'$) grew normally. As will be discussed, $20''+2R'+2RS/2BL'$ plants did not.

Various of these plants were then crossed with wheats containing defined grass clump dwarf genes: 'Gabo', homozygous for *D1* and *D3* (Hermsen 1967), and 'Spica', homozygous for *D2* and *D3* (McIntosh and Baker 1968). The F_1 progenies were grown in the greenhouse in 1981. F_2 progenies were grown on four occasions during 1982 but as greenhouse space was limiting, two sets of progenies were grown under field conditions which can only be described as severely stressed owing to drought.

Physiological and morphological studies of the seedling lethal phenotype were carried out at the Agricultural Research Institute, Wagga Wagga, and at the CSIRO Division of Plant Industry, Canberra. Rust screening was performed at the University of Sydney Plant Breeding Institute, Castle Hill, by Dr. R. A. McIntosh, Using rust strain 98-1,2,3,5,6 (Culture 781219) which is avirulent if the stem rust gene *Sr35* (=*SrTt1*) is present.

Results

Characterization of the seedling lethal phenotype

Seed containing the 2RS/2BL translocation as a disomic substitution are obtained by selfing $20''+2B'+2RS/2BL'$ heterozygotes. At normal temperatures, one quarter of the progeny of these heterozygotes consistently show seedling lethality, a ratio which indicates that the presence of the translocation does not affect the viability of the haploid gametes and that germination of the seed is not affected. The primary leaf emerges normally but leaf elongation immediately lags behind controls so that although up to four leaves may eventually be produced, and rarely secondary tillers, leaf and seedling growth ceases some fifteen to twenty days after imbibition when the seedlings have attained a maximum height of from 6–15 cm (Fig. 1a). During and after this period, the leaves gradually turn a darker green and then senesce with seedling death some 6 weeks postgermination.

Preliminary studies (data not shown) at cytological and physiological levels failed to determine a specific lesion in 2RS/2BL homozygous plants. Light microscopy revealed that cell division in the meristematic zones continued well after the lethal phenotype was apparent and electron microscopic studies could not distinguish any differences in the structure of nuclei, chloroplasts or mitochondria. When rates of photosynthesis were measured with an oxygen electrode, no differences were observed between the primary and secondary leaves of seedling lethals and controls. Also, $^{14}CO_2$-labelled photosynthate was shown to be transported from the leaves to the roots. When populations of seeds from the heterozygotes were grown in nutrient solution containing 10^{-5} M kinetin, indole acetic acid or gibberellic acid, none of these treatments overcame the seedling lethality.

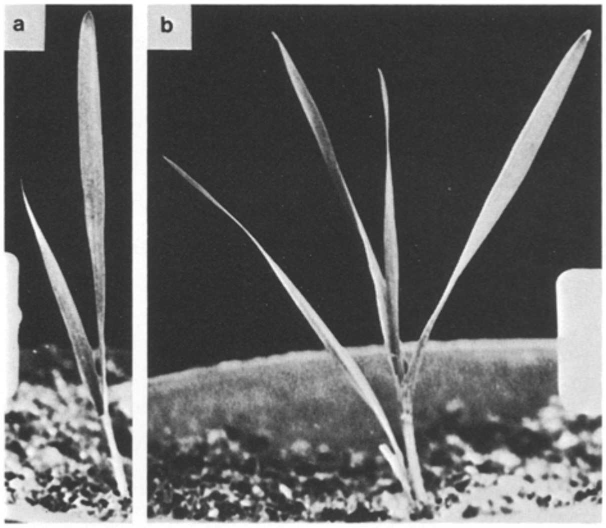

Fig. 1. A comparison between (**a**) a seedling lethal disomic for the translocation chromosome 2RS/2BL, and (**b**) a Type I grass clump dwarf from a cross between 'Gabo' and 'Spica' (two-thirds natural size)

The absence of a clear indicator as to the nature of the lethality associated with 2RS/2BL disomics led us to characterize this lethality at the genetic level.

Origin of the 2B chromosome involved in the translocation

Of the original wheats from which these lines were derived, only 'Timgalen' carries the stem rust resistance gene *Sr35* on chromosome 2B. The original parents and the lines derived from them in this breeding programme were screened with rust race 98-1,2,3,5,6 to determine whether any of the arms of chromosome 2B of Timgalen were involved in the 2B/2R translocation chromosomes (Table 1). These results show that *Sr35* is correlated with the presence of the short arm of chromosome 2B so that both 2B entire and the 2BS arm of the 2BS/2RL translocation have been introduced into these plants from 'Timgalen'. They also confirm that *Sr35* is lacking from 'Tr535'.

Table 1. Rust reaction and phenotype of the selfed progeny of parental wheats and derived lines

Parent[a]	No. and phenotype of progeny		
	Normal		Dwarfed
	Resistant (0; –; 1)	Suscep- tible (3+)	Suscep- tible
'Timgalen'	10	–	–
20″+2B″	10	–	–
20″+2BS/2RL″	9	–	–
20″+2B′+2BS/2RL′	9	–	–
'Tr535'	–	10	–
20″+2R″	–	10	–
20″+2R′+2BS/2RL′	8	2	–
20″+2B′+2R′	4	–	3[b]
20″+2B′+2RS/2BL′	4	–	6
20″+2BS/2RL′+2RS/2BL′	3	–	1

[a] Karyotypes determined with in situ hybridization

[b] The occurrence of dwarfs in this progeny is discussed later

New chromosomal combinations

Of the new combinations of chromosomes produced after the heterozygotes were intercrossed (see "Methods"), plants containing 20″+2R′+2RS/2BL′ were stunted, tillered profusely and were completely infertile. They appeared to be typical grass clump dwarfs. This raised the possibility that the seedling lethality shown by 2RS/2BL disomics could be an extreme form of grass clump dwarfism. We therefore investigated the effect of temperature on the growth of 2RS/2BL disomics. In duplicated experiments, we germinated and grew 20 progeny of 20″+2B′+2RS/2BL′ parents in growth cabinets maintained at 27 °C with a 16 h day/8 h night cycle. Approximately three-quarters of the plants grew normally while 11 of the 40 grew into infertile grass clump dwarfs. As this response showed some similarity with the response of wheat grass clump dwarfs to increased temperatures (Canvin and Evans 1963) and as these require the dwarfing gene *D1* in combination with both *D2* and *D3* or with *D2, D3* and *D4* (see "Discussion") it was clearly necessary to determine whether the gene causing seedling lethality interacted in any way with these dominant alleles.

Interaction of 2RS/2BL with wheat grass clump dwarf genes

The wheat cultivars 'Gabo' (homozygous for *D1* and *D3*) and 'Spica' (homozygous for *D2* and *D3*) were intercrossed with the lines produced in this breeding programme and with the original 'Chinese Spring' 2R(2B) substitution line. The F_1 progenies (Table 2) showed that the genotypes of 'Gabo' and 'Spica' were correct because their F_1 plants were all typical Type I grass clump dwarfs (Fig. 1 b) caused by the *D1d1D2d2-D3D3* genotype. Secondly, neither 'Timgalen' nor 'Tr535' has the *D2* allele and nor is this allele present on the 2BL arm of the 2RS/2BL translocation. Thirdly, and rather unexpectedly, the seedling lethality associated with 2RS/2BL did not interact with *D1* or any other of the dominant dwarfing alleles, at least as a heterozygote.

F_2 progeny screening

In the F_2 progenies, the presence or absence of 2BS/2RL and of the different 2B chromosomes was able to be determined by screening the progenies for the presence or absence of the rust resistance marker gene, *Sr35*. Thus, in crosses involving 20″+2B′+2RS/2BL′ or 20″+2BS/2RL′+2RS/2BL′ with Gabo or Spica, one half of the F_1 plants either 20″+2B″ or 20″+2B′+2BS/2RL′ were expected to give 100% normal F_2

Table 2. Phenotype of F_1 plants produced by intercrossing 'Gabo' and 'Spica' with relevant wheat cultivars and derived lines

Parental line[a]	No. and phenotype of F_1 progeny from crosses with	
	'GABO' (*D1D1d2d2D3D3*)	'SPICA' (*d1d1D2D2D3D3*)
'Gabo'	–	6 type I dwarfs
'Timgalen'	3 normal	4 normal
'Tr535'	4 normal	2 hybrid necrotics
20″+2B″	8 normal	4 normal
20″+2BS/2RL″	8 normal	4 normal
CS 2R(2B)	1 normal	1 normal
20″+2R″	7 normal	6 normal
20″+2B′+2RS/2BL′	37 normal	32 normal
20″+2BS/2RL′+2RS/2BL′	11 normal	16 normal

[a] Karyotypes determined with in situ hybridization

Table 3. F_2 progeny segregations

Parental karyotype[a]	Deduced karyotype of gamete transmitted to F_1 plant[b]		Segregation in F_2 of crosses with:									
			'GABO' Reaction[c]		Phenotype[d]			'SPICA' Reaction		Phenotype		
			R	S	N	D	L	R	S	N	D	L
20″+2B″	→ (20′+2B′)		19	8	27	0	0	26	5	31	0	0
	→ (20′+2B′)		92	16	128	0	0	283	96	567	0	0
20″+2B′+2RS/2BL′		Total	111	24	155	0	0	309	101	598	0	0
	→ (20′+2RS/2BL′)		0	41	199	14	52	0	424	829	17	29
	→ (20′+2RS/2BL′)		0	104	181	14	35	0	331	461	0	6
20″+2BS/2RL′+2RS/2BL′		Total	0	145	380	28	87	0	755	1290	17	35
	→ (20′+2BS/2RL′)		111	24	195	0	0	55	39	126	0	0
20″+2BS/2RL″	→ (20′+2BS/2RL′)		25	9	83	0	0	17	12	82	0	0
		Total	136	33	278	0	0	72	51	208	0	0
20″+2R″	→ (20′+2R′)		–	–	48	2	0	–	–	133	13	3
CS 2R(2B)	→ (20′+2R′)		–	–	260	31	7	–	–	257	31	6
		Total	–	–	308	33	7	–	–	390	44	9

[a] Karyotypes determined by in situ hybridization

[b] Karyotype of gametes from heterozygous parents deduced by segregation for rust resistance in F_2

[c] R = Rust resistant, S = susceptible

[d] N = Normal, D = dwarfed, L = seedling lethal

progenies segregating 3 : 1 for rust resistance while the other half 20″+2B′+2RS/2BL′ were expected to segregate 3 normal : 1 seedling lethal and be 100% susceptible to rust. The actual results are shown in Table 3. As predicted, those F_2 progenies which segregated for rust resistance produced no seedling lethals. Three of these four populations showed a good fit to the expected 3 : 1 ratio of resistant : susceptible plants. However, the F_2 populations involving 2B of 'Spica' with 2BS/2RL produced significantly fewer resistant plants than expected which indicated that in the respective F_1's, 2BS/2RL was transmitted with a lesser frequency (0.39) than was 2B entire (0.61). The critical data, however, relates to the progenies of those F_1 plants which contained 2B (from either 'Gabo' or 'Spica') and 2RS/2BL, recognized because all of their progeny were rust susceptible. None showed the expected 3 : 1 ratio of normals : seedling lethals and dwarfed plants also appeared. As compared to the seedling lethals, these dwarfs were characterized by at least a doubling of life span, increased tillering and greater numbers of leaves.

The low numbers of seedling lethals suggested that chromosome 2B entire had been transmitted preferentially to 2RS/2BL in the F_1 plants. As this was at variance with our previous observations on the transmission of these two chromosomes (May and Appels 1978, 1980), the chromosomes of a number of F_2 plants from 20″+2B′+2RS/2BL′ F_1 parents were labelled with the in situ probe (Table 4). The results of this study indicated that there was no significant reduction in the gametic transmission of the 2RS/2BL translocation which implies that the seedling lethality was being genetically suppressed. This was proven when we grew the seed: two of the six 'Gabo' and two of the three 'Spica' F_2 plants disomic for 2RS/2BL grew to maturity (Fig. 2).

Secondly, the unexpected recovery of dwarfed plants indicated that the seedling lethal phenotype was being

Table 4. Karyotypic identification of the progeny of F_1 plants (karyotype 20″+2B′+2RS/2BL′) in which chromosome 2B was introduced from a) 'Gabo', and b) 'Spica'

	No. of F_2 plants of karyotypes:		
	20″+2B″	20″+2B′+2RS/2BL′	20″+2RS/2BL″
a)	3	10	6
b)	6	3	3

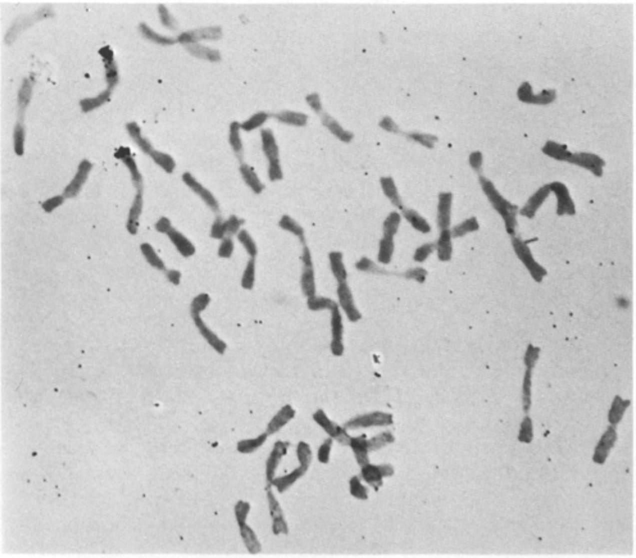

Fig. 2. The chromosomes of an F_2 plant from the cross between 'Gabo' and 20″+2B′+2RS/2BL′. This plant, disomic for 2RS/2BL (the 2RS arms are labelled by in situ hybridization) grew to maturity

further modified – probably by the environment in which these plants were grown. As noted previously, higher temperatures partially suppress the lethal phenotype and these F_2 progenies were grown in extremely hot conditions.

Lastly, Tables 2 and 3 show that when 'Gabo' and 'Spica' were crossed with lines in which 2R substitutes for 2B entire, the F_1 plants all grew normally but dwarfs and lethals were produced in the F_2. (These were also found when the progeny of 20″+2B′+2R′ plants were analysed for rust resistance). We believe that these are the result of de novo chromosomal rearrangements between 2R and 2B as a result of univalency of both of these chromosomes in the F_1 plants. This was postulated to be the origin of the translocated chromosomes in the original breeding programme (May and Appels 1978).

Discussion

The work which we have described in this paper has characterized the seedling lethality associated with the 2RS/2BL translocation chromosome. Whilst physiological and anatomical studies were unable to provide a clear lead in determining the nature of the seedling lethality, the habit of 20″+2R′+2RS/2BL′ plants, the effect of temperature on 2RS/2BL disomics and the discovery of dwarfed plants in the F_2 progenies showed that the seedling lethality was at least phenotypically related to the grass clump dwarf phenotype of wheat.

These were first described by Farrer (1898) but it was McMillan (1937) and Hermsen (1967) who developed the present classification of three grass clump dwarf types (Type I being the most severe) caused by various combinations of dominant dwarfing genes. Four genes have been implicated, *D1, D2, D3* and *D4* (for a recent discussion, see Worland and Law 1980). These have been located on three wheat chromosomes: *D1* and *D4* on 2DL (Hermsen 1963; McIntosh and Baker 1968; Silbaugh and Metzger 1970; Worland and Law 1980), *D2* on 2BL (Hurd and McGinniss 1958; McIntosh and Baker 1969) and *D3* on 4BL (Hermsen 1963). If we ignore recessive alleles, the gene combinations required to produce Type I dwarfs are *D1D2D3* or *D1D2D3D4*. Type II dwarfs require *D1D2D2, D1D2D4, D1D3D4* or *D1D4D4* (Hermsen 1967; Moore 1969; Worland and Law 1980). The presence of *D1* is essential.

The seedling lethal phenotype which we have described in this paper does not fit into the above grass clump dwarf categories because when we combined *D1* and *D3* or *D2* and *D3* in combination with 2RS/2BL, all of the F_1 plants grew normally. This indicated that the gene(s) causing seedling lethality did not interact genetically with the grass clump dwarf genes or at least as a dominant dwarfing gene would be expected to act when heterozygous. Is seedling lethality therefore, merely the result of homozygosity of a novel recessive gene on 2RS/2BL?

The F_2 results argue against this simple explanation in that 3:1 ratios of normals:lethals were not observed. Instead, in the 'Gabo' crosses involving 2RS/2BL, the combined F_2 data fit a ratio of 12 normals:1 dwarf:3 lethals ($\chi^2=0.849$; $0.5 > P > 0.3$) and when the karyotyped sibs of these progenies were grown, two of the six 2RS/2BL disomics grew to maturity. Such results are consistent with a two-gene epistatic interaction. A similar conclusion can be drawn from the first of the 'Spica' F_2 progenies in which 20″+2B′+2RS/2BL′ plants were the other parent. The F_1 plants of the same karyotype produced F_2 plants which segregated in the ratio of 60 normals:1 dwarf:3 lethals ($\chi^2=4.425$; $0.2 > P > 0.1$) and two out of three of the karyotyped 2RS/2BL disomics grew to maturity. These results are consistent with a three-gene epistatic interaction. However, the second 'Spica' F_2 progeny involving 2RS/2BL segregated in a 63 normal:1 lethal ratio ($\chi^2=0.234$; $0.2 > P > 0.1$).

These data indicate that the seedling lethality due to disomy of 2RS/2BL can be suppressed in a number of ways. Such differences have also been noted for grass clump dwarfs of wheat. For example, McMillan (1937) found that duplicate crosses between varieties could give different results so that crosses between strains occasionally needed consideration "rather than crosses between varieties". He also observed that "segregating generations may be affected markedly by the environment".

We conclude, therefore, that seedling lethality results from the interactions of a number of genes.

Whilst these have a combined effect which is at least partly analogous to the effects of the *D* genes which cause grass clump dwarfing in wheat, the seedling lethal genes do not interact genetically with these genes. As plants in which the 2R entire chromosome substituted for chromosome 2B do not show lethality, the seedling lethality associated with disomy of 2RS/2BL is clearly the result of the new combination of rye and wheat chromatin.

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