Theor Appl Genet (1998) 96: 1110–1120

A. J. Worland · V. Korzun · M. S. Röder
M. W. Ganal · C. N. Law

Genetic analysis of the dwarfing gene *Rht8* in wheat. Part II. The distribution and adaptive significance of allelic variants at the *Rht8* locus of wheat as revealed by microsatellite screening

Received: 17 October 1997/Accepted: 12 November 1997

Abstract Wheat microsatellite WMS 261 whose 192-bp allele has been shown to be diagnostic for the commercially important dwarfing gene *Rht8* was used to screen over 100 wheat varieties to determine the worldwide spread of *Rht8*. The results showed *Rht8* to be widespread in southern European wheats and to be present in many central European wheats including the Russian varieties 'Avrora', 'Bezostaya' and 'Kavkaz'. *Rht8* appears to be of importance to South European wheats as alternative giberellic acid (GA)-insensitive dwarfing genes do not appear to be adapted to this environment. The very successful semi-dwarf varieties bred by CIMMYT, Mexico, for distribution worldwide have been thought to carry *Rht8* combined with GA-insensitive dwarfing genes. Additional height reduction would have been obtained from pleiotropic effects of the photoperiod-response gene *Ppd1* that is essential to the adaptability of varieties bred for growing under short-winter days in tropical and sub-tropical areas. The microsatellite analysis showed that CIMMYT wheats lack *Rht8* and carry a WMS 261 allelic variant of 165 bp that has been associated with promoting height. This presumably has adaptive significance in partly counteracting the effects of other dwarfing genes and preventing the plants being too short. Most UK, German and French wheats carry an allelic variant at the WMS 261 locus with 174 bp. This could be selected because of linkage with the recessive photoperiod-sensitive *ppd1* allele that is thought to offer adaptive significance northern European wheats.

Communicated by G. Wenzel

A. J. Worland (✉) · C. N. Law
John Innes Centre, Cereals Research Department, Norwich,
NR4 7UJ, UK
Fax: + 44 1603 502241

V. Korzun · M. S. Röder · M. W. Ganal
Institut fur Pflanzengenetik und Kulturpflanzenforschung (IPK),
Corrensstr 3, D-06446, Gatersleben, Germany

Key words Adaptability · Dwarfing gene (*Rht8*) · Microsatellite · Molecular markers · Wheat

Introduction

A semi-dwarf stature for wheat (*Triticum aestivum* $2n = 6x = 42$) has been shown to permit increased yields through more efficient utilisation of available assimilates (Ford et al. 1985) and by reducing yield losses associated with crop lodging (Dalrymple 1986).

The very complex genetic control of plant height in wheat is determined by genes on many chromosomes (Law et al. 1973). To date 21 genes of major effect on plant height have been assigned *Rht* symbols in wheat (McIntosh et al. 1995). The major genes determining plant height are normally classified into two groups depending on their reaction to exogenous giberellic acid (GA) (Gale and Gregory 1977; Börner et al. 1996). The GA-insensitive dwarfing genes of wheat are present in two homoeologous allelic series located on the short arms of chromosomes 4B[1] and 4D (Gale et al. 1975; Gale and Marshall 1976; McVittie et al. 1978). GA-responsive dwarfing genes have been located on chromosomes 2A (Worland et al. 1980), 2DS (Worland and Law 1986), 7BS (Worland et al. 1990) and 5A (Sutka and Kovacs 1987). The height reductions determined by the wheat dwarfing genes can be further modulated by increasing the potency of alleles for height suppression (chromosome 4B[1] and 4D, GA insensitive dwarfing genes, *Rht12*), reducing the potency of height-promoting alleles (*Rht7*, *Rht8*) or by shortening the life cycle (pleiotropic effects of *Ppd1*, *Ppd2* or *Ppd3*).

In bread wheat there is a general correlation between reduced height and reduced yield (Law et al. 1973). In

---

[1] Nomenclature post-7th International Wheat Genetics Symposium, Cambridge, UK, 1988

order for a height-reducing gene to be of commercial significance it must either break the height/yield correlation and combine reductions in height with increases in yield or at a minimum must achieve the height reduction without influencing yield so that agronomic advantages may be achieved by reduced lodging. Few major dwarfing genes are known to break the correlations between reduced height and reduced yield. The most important group of genes breaking this correlation are the group 4 GA-insensitive dwarfing genes, in particular *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) derived from 'Norin 10' and *Rht-B1d* (*Rht1*-'Saitama 27' allele) derived from 'Saitama 27' (Gale and Youseffian 1985; Worland and Petrovic 1986) (new nomenclature after Börner et al. 1996). Under optimal circumstances in the UK, *Rht-B1b* or *Rht-D1b* can combine height reductions of approximately 16% with similar levels of increased yield. These GA-insensitive dwarfing genes are probably present in around 90% of the world's semi-dwarf wheat crop and were responsible for the worldwide green revolution in wheat cultivation (Borlaug 1968). Under certain circumstances where warm temperatures occur around the time of meiosis, interactions between these dwarfing genes and the environment can cause fertility reductions and loss of yield advantages (Law and Worland 1985). It is therefore of upmost importance that alternative GA-responsive dwarfing genes are studied and that where these offer potential to improving crop yields, molecular markers should be generated to enable breeders to recognise and select for these genes in their segregating populations.

The most important of the GA-responsive dwarfing genes and those of proven commercial potential are derived from the Japanese variety 'Akakomugi' and are widespread in southern European wheats where environmental interactions restrict the potential of the GA-insensitive dwarfing genes (Worland et al. 1990). Two important dwarfing genes, *Ppd1* and *Rht8*, were introduced from 'Akakomugi' into European wheats in the 1930s by the Italian breeder Strampelli (Strampelli 1932). Of these 2 genes, *Ppd1* is really a gene for insensitivity to photoperiod that reduces plant height by shortening the life cycle (Worland and Law 1986). This gene and its homoeologues, *Ppd2* and *Ppd3*, are very important to climatic adaptability (Worland 1996). Although no readily detectable molecular marker is at present available for *Ppd1*, its presence in varieties can be detected by growing plants under short-day conditions (Worland et al. 1994).

Whilst *Ppd1* has proven extremely important in promoting a reduction in height and increases in adaptability and yield in southern Europe and in the spring wheats introduced worldwide by CIMMYT, its mode of action by accelerating the number of days to flowering restricts its use to those environments where earlier flowering and a restriction in the life cycle can be accepted. In areas such as much of northern Europe and America maximal yields are associated with extended life cycles so *Ppd* genes cannot be utilised to reduce plant height (Worland et al. 1997).

At present the only viable alternative major height-reducing gene of wheat other than the GA-insensitive or the photoperiod-insensitive genes is probably *Rht8*. *Rht8* has been shown to be a weak allele at a locus on chromosome 2DS of genes for height promotion (Worland et al. 1990). The presence of *Rht8* in a variety can only unequivocally be established by developing or field-selecting monosomics for the 2D chromosome that carry the gene. Normally all group 2 chromosomes carry strong alleles for promotion of plant height. In the monosomic conditions, at reduced dosage, they are less efficient at promoting plant height and, consequently, group 2 monosomics are phenotypically shorter than their parental variety (Table 1). The reduction in height associated with the loss of a group 2 chromosome is normally in excess of 20%. Height reduction associated with the weaker height-promoting allele *Rht8* is however less than 10% (Worland et al. 1992). Monosomic series are at present available in around 70 varieties worldwide (Worland 1988). Observations on the height of group 2 monosomics compared to that of the parental varietal have indicated that the varieties 'Mara', 'Sava', and 'N.S. Rana' carry *Rht8* on their 2D chromosome. They also indicate the probable presence of homoeologous genes on chromosome 2A of 'Carola' and chromosome 2B of 'Chinese Spring' (Table 1). Height observations are, however not available for most monosomic series. Additional varieties could be screened for the presence of *Rht8* on chromosome 2D by carrying out reciprocal monosomic analysis between the variety and established group 2 monosomics (Snape and Law 1980). Such analysis was used by Snape to show that the variety 'Talent' does not carry *Rht8* (personal communication).

Table 1 Height[a] of group 2 monosomics from a range of wheat varieties expressed as a percentage of the euploid varietal control

Variety	Chromosome			Translocations
	2A	2B	2D	
Bersee	75.1	73.9	74.3	
Caribo	74.9	89.2	81.3	
Carola	93.6	79.9	84.0	
Chinese Spring	83.0	92.6	81.7	
Cappelle-Desprez	76.3	76.1	77.5	
Hobbit Sib	76.5	74.0	78.0	
Mara	80.4	82.8	90.9	
N.S. Rana	78.2	76.9	90.7	
Poros	78.7	74.9	86.7	7B-2D
Probus	75.9	–	79.9	
Rannyaya 12	77.8	80.3	88.0	1B-2D
Sava	78.6	80.2	92.8	
Zlatka	91.8	80.6	83.2	

[a] Height data from pot-grown plants were supplied by members of the European Wheat Aneuploid Cooperative (EWAC Newsl 1992)

The difficulty in recognising *Rht8* in varieties has led to many claims concerning the distribution of the gene worldwide and its potential benefits to breeding programmes. These claims are difficult to substantiate. *Rht8* has been reported to be present in Chinese and CIMMYT varieties (Mishra and Kushwaha 1995) and to enhance the yield of these wheats. In reality the only definitive studies on the effect of *Rht8* involve the use of precise genetic stocks derived from varieties like 'Mara' from Italy and 'Sava' from Yugoslavia where both pedigree analysis and observations on defined aneuploid stocks confirm that *Rht8* must be present on the 2D chromosome. The data from these studies are restricted to both a limited range of varieties and limited array of environments. These studies suggest that *Rht8* reduces height by around 8–10 cm in the UK (Worland and Law 1986; Worland et al. 1988a,b), 5 cm in mid Germany (Worland et al. 1992) and 5–7 cm in Yugoslavia (Worland et al. 1988a, b, 1990). In all cited examples, data were obtained on single-chromosome recombinant lines between chromosomes 2D of 'Mara' (*Rht8*) and 'Cappelle-Desprez' (*rht8*) in a homozygous 'Cappelle-Desprez' background. Few significant additive genotypic effects of *Rht8* were detected on other agronomic characters. Interactions were detected between *Rht8* and *Ppd1* for spikelet numbers, grain size and ear yield (Worland et al. 1988a, b). No interactive environmental effects were detected for *Rht8* when similar lines were tested in England and Yugoslavia. These results suggest that at least in Europe, within the varietal background, year and environment limitations of the trials *Rht8* could be used to reduce height without adverse effect on plant yield.

Recently a microsatellite marker, WMS 261, has been identified that shows very restricted recombination with *Rht8* (Korzun et al. 1998). Three main allelic variants of 165 bp, 174 bp and 192 bp have been detected at the WMS 261 locus on the short arm of chromosome 2D. The 165-bp variant was found to be diagnostic for the CIMMYT variety 'Ciano 67', the 174-bp variant diagnostic for 'Cappelle Desprez', the tall control variety in experiments determining the pleiotropic effects of *Rht8,* and the 192-bp variant diagnostic for the Italian variety 'Mara', the donor of *Rht8*. Genetic analysis of single-chromosome recombinant lines developed in a 'Cappelle-Desprez' background between 2D chromosomes of 'Cappelle-Desprez' and 'Ciano 67' shows a significant height increase of around 3–4 cm associated with the WMS 261 165-bp allele compared to the WMS 261 174-bp allele. This 3- to 4-cm increase is in addition to the 5–10 cm of the WMS 261 192-bp allele versus WMS 261 174-bp allele comparison.

It is anticipated that the very close linkage of WMS 261 to *Rht8* will permit the use of the microsatellite as a marker for determining the distribution of *Rht8* in international breeding programmes and to demonstrate how *Rht8* has been transmitted from initial crosses involving the source variety 'Akakomugi'. In the experiments described here over 100 varieties have been screened for allelic variants at the WMS 261 locus. The varieties were chosen to include key varieties in the pedigrees of modern varieties that are thought to carry *Rht8*, varieties from diverse international breeding programmes and check varieties that have been shown by cytological observations to carry *Rht8* and are therefore able to be used to verify that recombination has not occurred between WMS 261 and *Rht8* and thus verify conclusions drawn from the marker association.

Materials and methods

Varieties

A diverse array of international varieties listed in Table 2 were chosen for microsatellite screening. The majority of the seed stocks were obtained from John Innes Centre germplasm collection. Additional key varieties were obtained from the IPK genebank, Gatersleben, Germany.

Methods

The methods used for determining the allelic variant at the WMS 261 microsatellite locus are fully described by Korzun et al. (1998). For varietal analysis, DNA was extracted from one to five grains using procedures described by Plaschke et al. (1995).

Results and discussion

The majority of the varieties screened for WMS 261 fell into three groups (Table 2) on the basis of their containing either the 165-bp fragment characteristic of 'Ciano 67' (28 lines), the 174-bp fragment characteristic of 'Cappelle-Desprez' (25 lines) or the 192-bp fragment characteristic of 'Mara' (58 lines). Seven varieties carried novel bands of, in each case, more than 200 bp. An additional 6 varieties carried two or three fragments with different numbers of base pairs. Screening individual seed from sample varieties within this group indicated that the pooled DNA used in the original screen carried more than one fragment due to a heterozygous stock indicating either residual heterozygosity in the original variety or contamination problems during stock maintenance. Varieties carrying more than one WMS 261 allelic variant were removed from further analysis.

Correlation of the diagnostic microsatellite marker with *Rht8* in control varieties

For the wheat microsatellite allele WMS 261-192 bp to be successfully used as a diagnostic marker for *Rht8* it must be shown that the tight linkage in the source

Table 2 Classification of wheat varieties for allelic variants of microsatellite WMS 261

Variety	Origin	Photoperiod[a] response	GA[b] response
WMS 261 (165-bp fragment)			
Autonomia	Italy	I	R
Bob white	Mexico	R	*Rht-B1b*
Brevor	USA	–	R
Chaimite	Portugal	I	*Rht -B1d*
Ciano 67	Mexico	I	*Rht-D1b*
Chris	USA	I	R
Federation	Australia	–	R
Frontana	Brazil	I	R
Glennson 81	Mexico	I	*Rht-B1b*
Jupateco 73	Mexico	I	*Rht-B1b/Rht-D1b*
Kenya	Kenya	–	R
Klein 32	Argentina	I	–
Lerma Rojo	Mexico	I	*Rht-B1b*
Lusitano	Portugal	–	–
Maringa	Brazil	I	R
Mentana	Italy	I	R
Nainari 60	Mexico	I	R
New Thatch	USA	R	R
Opata 85	Mexico	I	*Rht-B1b*
Othello	Germany	–	–
Penjamo 62	Mexico	I	*Rht-B1b*
Quaderna	Italy	I	R
Rex	France	–	–
Riete	Italy	R	R
Saitama 27	Japan	I	*Rht-B1d*
Spica	Australia	I (*Ppd2*)	R
Veery's	Mexico	I	*Rht-B1b*
Victo	France	I	*Rht-B1b*
WMS 261 (174-bp fragment)			
Balkan	Bulgaria	I	R
Balkan	Yugoslavia	I	R
Bunyip	Australia	–	–
Cappelle-Desprez	France	R	R
Eureka	France	I	*Rht-B1b* or *Rht-D1b*
Festival	France	I	R
Fronteira	Brazil	I	R
Fultz	USA	–	–
Gabo	Australia	I	R
Heine VII	Germany	–	–
Inallettabile 95	Italy	–	–
Jena	France	–	–
Klein Rendidor	Argentina	I	R
Leonardo	Italy	I	R
Lutescens 17	Russia	–	–
Mironovskaya 808	Ukraine	R	R
Norin 10	Japan	R	*Rht-B1b* + *Rht-D1b*
Norin 10/ Brevior 14	USA	–	*Rht-B1b* + *Rht-D1b*
Podunavka	Yugoslavia	–	–
Record	Australia	–	–
Red Coat	USA	–	–
Soissons	France	I	*Rht-B1b*
Talent	France	I	R
Tevere	Italy	–	–
Timstein	Australia	I	R
Wilhelmina	Netherlands	R	R
WMS 261 (192-bp fragment)			
Alfa	Poland	R	*Rht-B1d*
Aquila	Italy	–	R
Ardito	Italy	I	R
Argelato	Italy	I	*Rht-B1d*
Avrora	Russia	I	R
Beauchamps	France	I	R
Bezostaya	Russia	I	R

Table 2 Continued

Variety	Origin	Photoperiod response	GA response
Biserka	Yugoslavia	I	R
Campodoro	Italy	I	R
Centauro	Italy	I	*Rht-B1d*
Chikushi-Komugi (Norin 121)	Japan	–	*Rht-B1b*
Damiano	Italy	I	R
Dneprovskaya	Ukraine	–	R
Etoile-de-choisy	France	I	R
Etruria	Italy	I	*Rht-B1d*
Fakuho-Kumugi (Norin 124)	Japan	–	*Rht-B1d*
Farnese	Italy	I	*Rht-B1d*
Favorite	Romania	–	R
Fiorello	Italy	I	R
Fortunato	Italy	I	R
Funo	Italy	–	R
Gala	France	I	R
Haya Komugi	Japan	–	*Rht-D1b*
Impeto	Italy	I	R
Irnerio	Italy	I	R
Jugoslavia	Yugoslavia	I	R
Kavkas	Russia	I	R
Libellula	Italy	I	R
Lovrin 32	Romania	–	*Rht-B1b*
Mara	Italy	I	R
Marzotto	Italy	–	R
Neretva	Yugoslavia	–	R
Nizija	Yugoslavia	I	R
N.S. Rana 1	Yugoslavia	I	R
N.S. Rana 2	Yugoslavia	I	R
N.S. 649	Yugoslavia	I	R
N.S. 3014	Yugoslavia	I	R
Orlandi	Italy	I	*Rht-B1d*
Partizanka	Yugoslavia	I	R
Partizanka Niska	Yugoslavia	I	R
Posavka 2	Yugoslavia	I	R
Pomoravka	Yugoslavia	I	R
Produttore	Italy	I	*Rht-B1d*
Radusa	Yugoslavia	–	*Rht-B1b* or *Rht-D1b*
Salto	Italy	I	R
Sanja	Yugoslavia	I	R
San pastore	Italy	I	R
Sava	Yugoslavia	I	R
Siette Cerros	Mexico	I	*Rht-B1b*
Sinvaloche	Argentina	I	R
Skopljanka	Yugoslavia	I	*Rht-B1d*
Skorospelka 3B	Russia	I	R
Superzlatna	Yugoslavia	I	*Rht-B1d*
Transilvania	Romania	I	*Rht-B1b* or *Rht-D1b*
Una	Yugoslavia	I	R
Villa Glori	Italy	–	R
Zitnica	Yugoslavia	I	R
Zvezda	Yugoslavia	I	R
WMS 261 (201-bp fragment)			
Pliska	Bulgaria	I	*Rht-B1b* or *Rht-D1b*
Courtot	France	I	*Rht-B1b* + *Rht-D1b*
WMS 261 (210-bp fragment)			
Chino	Argentina	–	–
Klein Esterello	Argentina	R	R
Klein 157	Argentina	R	R
WMS 261 (215-bp fragment)			
Klein 49	Argentina	R	R

[a] I – photoperiod insensitive; R – photoperiod responsive

[b] R – GA responsive

variety 'Akakomugi' is not broken during varietal selection. To demonstrate this, we include varieties that can by pedigree analysis and by analysis of precise genetic stocks, be definitively shown to carry *Rht8* in the varietal screen. The varieties 'Mara', 'N.S. Rana' and 'Sava' that carry *Rht8* as recognised by height comparisons between chromosome 2D monosomics and euploid controls (Table 1) all carried the diagnostic WMS 261-192-bp microsatellite allele. Conversely, the allele has not been detected in control varieties where *Rht8* would not be expected by pedigree analysis or by monosomic comparisons.

Worldwide distribution of WMS 261 allelic variants

The dwarfing genes credited with playing major roles in improving wheat yields *(Rht-B1b*, *Rht-B1d*, *Rht-D1b,* *Rht8*, Ppd1) all seem to have either originated in Japan or to have been incorporated into early Japanese varieties before spreading into worldwide breeding programmes. The tests carried out here confirmed that 'Akakomugi', the source variety for *Rht8*, carries the diagnostic WMS 261-192-bp microsatellite. Of 5 other Japanese varieties tested, 2 modern varieties 'Chikushi-Komugi' ('Norin 121') and 'Fakuho-Komugi' ('Norin 124') both carry the *Rht8* allele, as does 'Haya-Komugi', an old land-race and a parent of another important Japanese dwarfing source variety 'Saitama 27'. 'Saitama 27' itself has not obtained the diagnostic *Rht8* allele from 'Haya-Komugi' but carries the WMS 261-165-bp allele. The third important Japanese dwarfing gene source variety, 'Norin 10', carries the WMS 261-174-bp allele. The results therefore indicate that even in the early decades of the twentieth century all three major WMS 261 alleles were segregating in Japanese wheats.

Varietal pedigrees indicate that the primary route for the worldwide spread of *Rht8* from the Japanese source variety 'Akakomugi' was through the Italian breeding programmes of Strampelli. In a crucial cross made by Strampelli around 1925, 'Akakomugi' was crossed onto a hybrid between the Dutch variety 'Wilhelmina' and the Italian landrace 'Riete' (Fig. 1). From this key cross Strampelli selected the varieties 'Villa Gloria', 'Damiano', 'Mentana' and 'Ardito', that combined the short-straw, early-maturity and high-yield potential of 'Akakomugi' with the adaptability of local varieties (Strampelli 1932). The 4 derivative varieties then became important parents in breeding modern Italian wheats, Yugoslavian wheats (Fig. 1) (Borojevic and Potocanac 1966), Mexican CIMMYT varieties (Fig. 2)

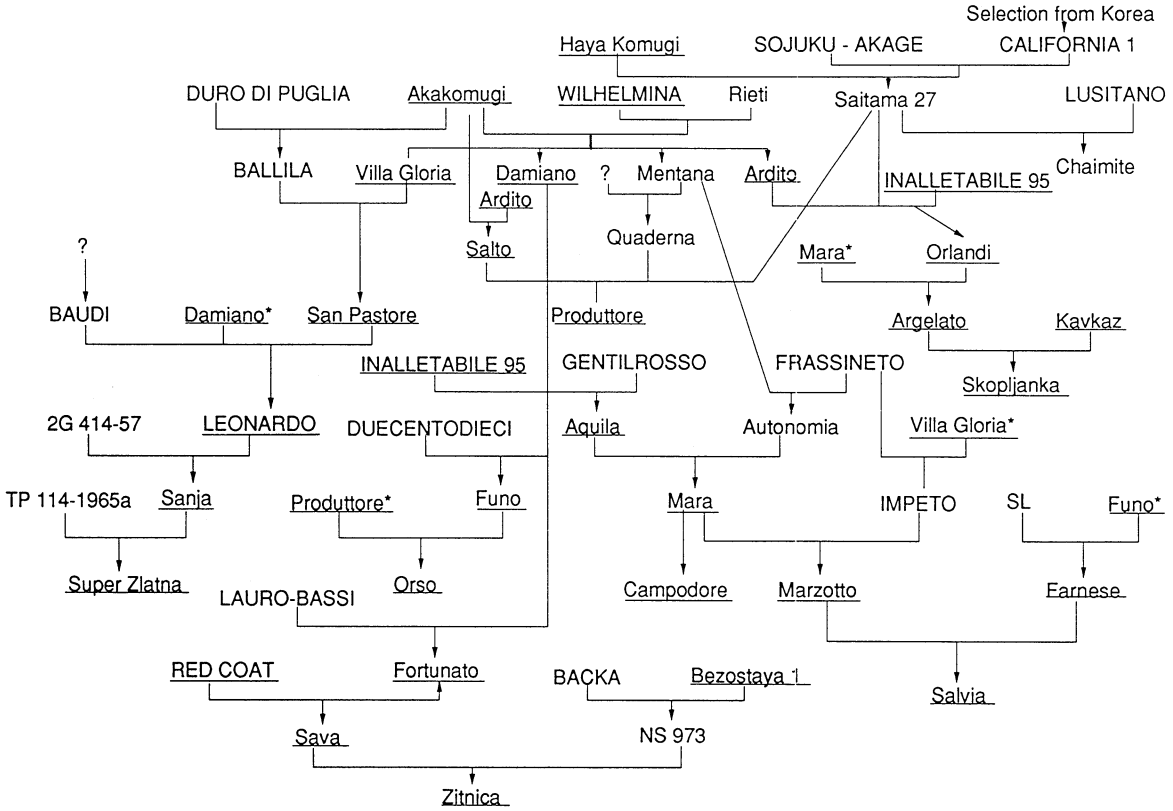


Fig. 1 Pedigree and WMS 261 classification of South European wheat varieties: *capital letters* untested, *capital letters underlined* WMS 261-174 bp, *small-case letters* WMS 261-165 bp, *small-case letters underlined* WMS 261-192 bp. Those varieties indicated by an *asterisk* (*) have their pedigree given elsewhere

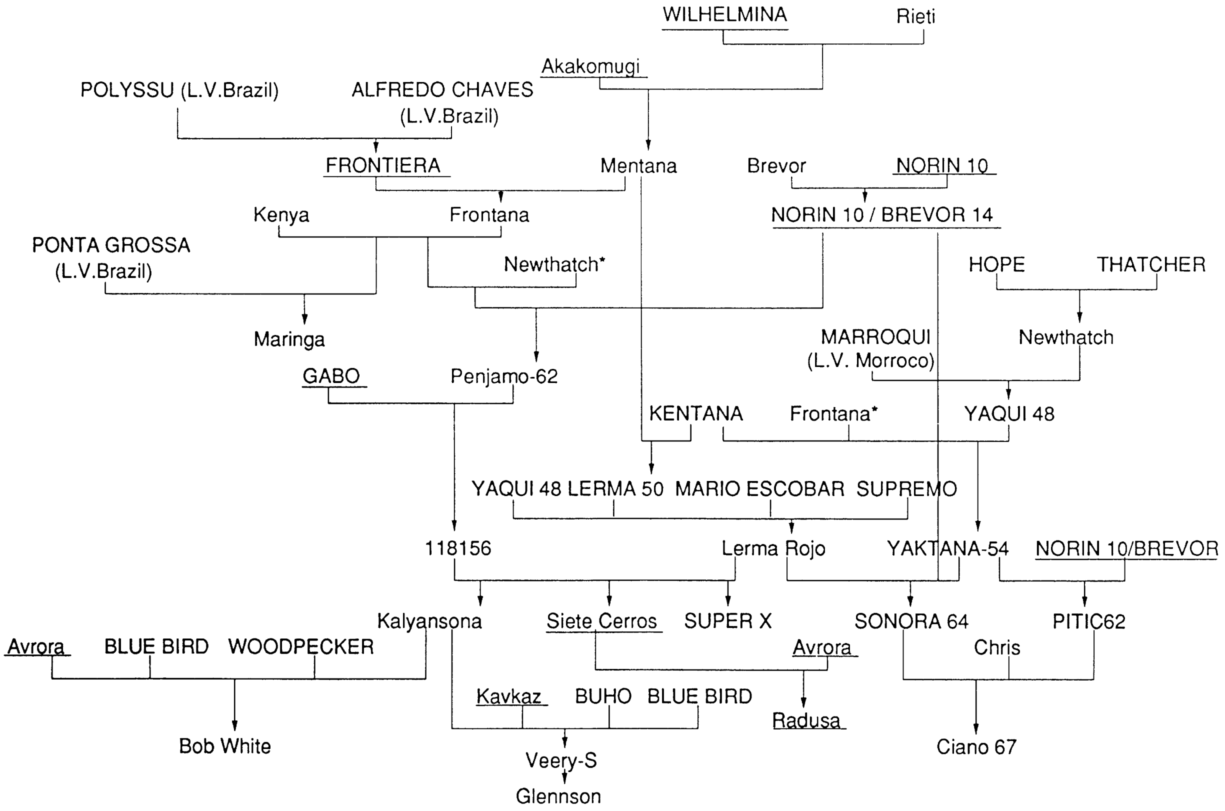


Fig. 2 Pedigree and WMS 261 classification of CIMMYT, Mexican wheat varieties. Code for varieties as in Fig. 1

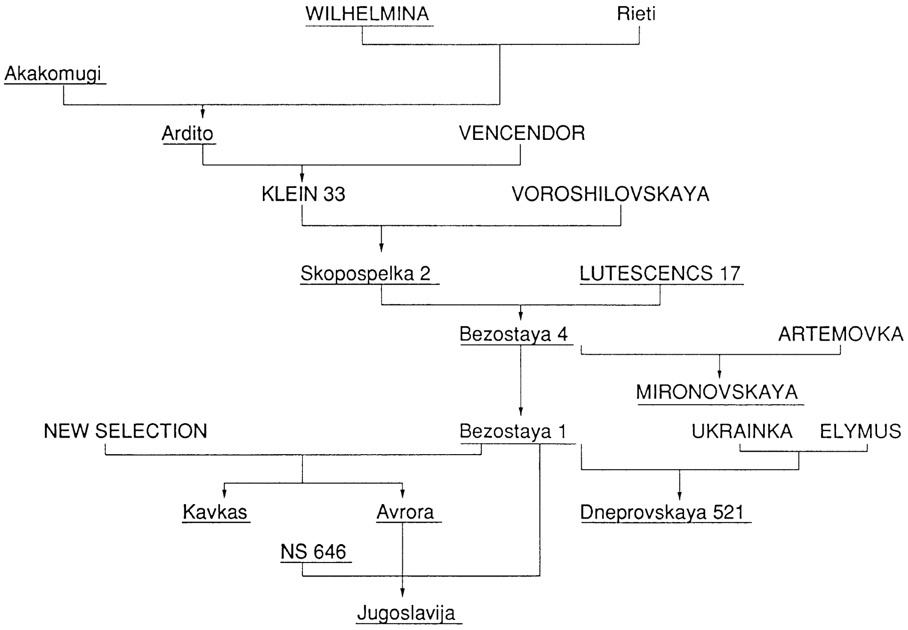
(Borlaug 1968) and Russian varieties (Fig. 3). Tests carried out here to determine the presence of the WMS 261 microsatellite allele present in Strampelli's key Italian wheat varieties show that the three parents of Strampelli's original cross each display different WMS 261 alleles with 'Akakomugi' carrying WMS 261-192 bp; 'Wilhelmina', WMS 261-174 bp; 'Riete', WMS 261-165 bp. Of the 4 varieties derived from Strampelli's initial cross all except 'Mentana' carried the diagnostic *Rht8* (WMS261-192-bp) allele; 'Mentana' carried the WMS 261-165-bp allele.

Pedigree analysis of a wide range of Italian and Yugoslavian varieties derived from offspring of Strampelli's original cross (Fig. 1, Table 2) show that all tested modern cultivars carry the diagnostic allele of *Rht8*. The WMS 261-165-bp allele occurs only in early generation derivatives of 'Mentana'.

In northern Italy a separate breeding programme of the Production Sementi Seed Company of Bologna utilised 'Saitama 27' as a source of Japanese genes for short straw and early flowering. Until the present microsatellite analysis it was uncertain whether 'Saitama 27', like 'Akakomugi', carried *Rht8* linked to its *Ppd1* gene for early maturity on the short arm of chromosome 2D. Our analyses indicate that 'Saitama 27' carries the WMS 261-165-bp allele and thus would not have been an alternative source for the introduction of *Rht8* into Italian wheat germplasm. The results (Table 2, Fig. 1) indicate that as with derivatives of 'Mentana' the WMS 261-165-bp allele was not selected in southern European derivative varieties following hybridisation to cultivars carrying *Rht8*. This clearly indicates a selective advantage of *Rht8* in southern European breeding programmes.

A second major area for the possible spread of *Rht8* are the CIMMYT green revolution-wheat improvement programmes. In these programmes Borlaug (1968) combined the semi-dwarfing genes *Rht-B1b* and *Rht-D1b* derived via Vogel's American variety 'Norin 10/Brevor 14' with *Ppd* genes for photoperiod insensitivity and early maturity derived from Italian varieties. Our investigations show that 'Norin 10/Brevor 14' carries the WMS 261-174-bp allele and has not been a source of *Rht8*. The Italian wheats used by Borlaug reached Mexico by two separate routes (Fig. 2). The main route was probably via South America with 'Mentana' being introduced into Brazil where it was crossed with Brazilian landraces to develop 'Frontana' and later 'Maringa'. Both these varieties carry the same WMS 261-165-bp allele as 'Mentana'. 'Frontana' or its derivative variety 'Yaktana 54' were crossed with 'Norin 10/Brevor 14' to develop early-maturing

Fig. 3 Pedigree and WMS 261 classification of wheat varieties from the former Soviet Union. Code for varieties as in Fig. 1



semi-dwarf CIMMYT varieties for their worldwide programmes of germplasm improvement. 'Mentana' was also used directly in breeding other early CIMMYT varieties. All early CIMMYT varieties tested for WMS 261 alleles carry the same 165-bp allele as 'Mentana'. Following the initial development of CIMMYT wheats several crosses were made to the Russian varieties 'Avrora' and 'Kavkaz' that carry beneficial genes for disease resistance on a translocated 1B/1R rye chromosome introduction. Both 'Avrora' and 'Kavkaz' carry the WMS 261-192-bp band diagnostic for *Rht8* and could have introduced this into CIMMYT wheats. All of the tested progeny of crosses introgressing genes from the Russian varieties into CIMMYT germplasm lack the microsatellite allele diagnostic for *Rht8* but carry the 165-bp allele derived from 'Mentana' suggesting an adaptive significance for this allele in CIMMYT germplasm. Our analysis suggests 'Siette Cerros' carries the *Rht8* WMS 261-192-bp allele, but the pedigree of this line suggests that the tested stocks or varietal pedigree are incorrect. Interestingly, a Yugoslavian spring variety, 'Radusa', derived from a cross of 'Siette Cerros' with 'Avrora' carries the *Rht8* allele. This could be derived from 'Avrora' and might offer adaptive advantages under the differing climatic conditions of Yugoslavia.

We have mentioned that the Russian varieties 'Avrora' and 'Kavkaz' carry the microsatellite allele diagnostic for *Rht8*. Pedigree analysis (Fig. 3) show that important Russian wheat varieties can be traced to Strampelli's variety 'Ardito'. 'Ardito' was bred in 1916 and by 1926 occupied nearly all of the 500,000 hectares of early-maturing wheat varieties grown in Italy (Strampelli 1932). 'Ardito' was personally taken to Argentina by Strampelli in 1925 and crossed there to develop relatively unsuccessful short-strawed Argentinian wheats like 'Klein 33' (O. Klein, personal comunication). 'Klein 33' later became a major progenitor to the improvement of Russian wheats (Dalrymple 1986), introducing *Rht8* into key Russian varieties including one of the most universally popular varieties, 'Bezostaya'. The allele is not present in 'Mironovskaya 808' that was selected for cold tolerance. *Rht8* has also been transmitted via 'Bezostaya' into Yugoslavian varieties like 'Jugoslavia'.

Our survey did not test Chinese wheats that are often quoted as carriers of *Rht8* (Dalrymple 1986; Mishra and Kushwaha 1995). From the varieties tested there is no evidence of *Rht8* in Australian or North American wheat varieties. From pedigree analysis, cytological analysis and the limited range of varieties tested here there is no evidence for *Rht8* in UK or German wheats. *Rht8* has, however, been incorporated into a few French wheats by linkage to *Ppd1*. WMS 261 screening of photoperiod-insensitive French wheats shows that 4 varieties ('Eureka', 'Festival', 'Soissons' and 'Talent') carry the 174-bp allele, 3 varieties ('Beauchamps', 'Etoile-de-Choisy' and 'Gala') the 192-bp *Rht8* allele, 1 variety, 'Victo', the WMS 261-165-bp allele and that 'Courtot' carries a unique WMS 261-202-bp allele. The 3 varieties carrying the *Rht8* allele are the oldest of the French varieties tested. 'Etiole-de-Choisy', released in 1950, would have obtained *Rht8* from the parental variety 'Ardito'. 'Beauchamps' and 'Gala' are both derived from crosses involving 'Etiole-de-Choisy'. Of the 5 varieties carrying the WMS 261-174-bp allele, 'Talent' would have obtained its photoperiod insensitivity from 'Fortunato', but recombination must have separated *Ppd1* from *Rht8* with 'Talent' carrying the microsatellite allele present in 'Cappelle-Desprez' and traditional photoperiod-sensitive French wheats. 'Festival' is derived from 'Talent'. 'Soissons' has the photoperiod-insensitive variety 'Courtot' and photoperiod-insensitive Mexican wheats in its parentage, neither of which combine *Ppd1* with WMS 261-174 bp suggesting again a selective advantage for breaking the linkage. The origin of photoperiod insensitivity in 'Eureka' is not obvious from its stated pedigree. 'Victo' is a derivative of North American breeding programmes and would probably have obtained its photoperiod insensitivity and WMS 261-165-bp allele from parental CIMMYT varieties.

The results of the varietal analysis with wheat microsatellite WMS 261 described above would suggest that the distribution of allelic variants in international breeding programmes is more likely due to selection than chance. The main areas of distribution for the WMS 261 allele diagnostic for *Rht8* are throughout southern Europe and throughout the main Russian breeding programmes. CIMMYT varieties carry the WMS 261-165-bp microsatellite allele, whilst the standard WMS 261-174-bp allele appears to be present in most of the UK, German and French varieties tested.

The vast majority of the varieties tested in the microsatellite analysis are photoperiod-insensitive (Table 2), with the insensitivity presumably due to *Ppd1* that is closely linked to *Rht8* in the parental variety 'Akakomugi'. It is clear that linkage between the two genes can be broken, as occurred in 'Mentana', 1 of 4 varieties selected from Strampelli's original 'Akakomugi' cross. Most southern European breeders have been interested in improving the adaptability of their varieties by reducing plant height, accelerating the time to flowering to improve grain fill before the onset of desiccating summer conditions and to increase spikelet fertility and consequently yield (Borojevic and Potacanac 1966). All these characteristics can be obtained by introducing the *Ppd1*/*Rht8* linkage group into varieties (Worland et al. 1988a). The alternative route for height reduction and increase of spikelet fertilities is to introduce GA-insensitive dwarfing genes such as *Rht-B1b* or *Rht-D1b* (Gale and Yousefian 1985). These genes however appear to exhibit deleterious environmental interactions that inhibit their use in southern European breeding programmes (Worland et al. 1988b). The *Ppd1*/*Rht8* linkage group on chromosome 2D would therefore offer southern European breeders the best opportunities for breeding high-yielding, early-maturing, semi-dwarf wheats and would explain why it has been selected in the Yugoslavian and Italian breeding programmes. Similar selection criteria could be important in the areas of the former Soviet Union where the Russian varieties 'Bezostaya', 'Avrora' and 'Kavkas' have been so successful by their wide adaptability.

The presence of the WMS 261-165-bp allele in modern CIMMYT wheats can probably be explained by the association of this allele with a significant increase in plant height (Korzun et al. 1998). All modern CIMMYT wheats rely heavily on the 'Norin 10' dwarfing genes *Rht-B1b* and *Rht-D1b,* singly or in combination, to reduce height and increase yield through improved spikelet fertilities and lodging resistance (Rajaram and Van Ginkel 1996). The CIMMYT spring wheats also carry photoperiod-insensitive genes to permit flowering when plants are grown during the short-day winter season. A pleiotropic effect of the photoperiod insensitivity is to further reduce plant height (Worland et al. 1988a). It is probable that in CIMMYT wheats, a combination of GA-insensitive dwarfing genes plus *Ppd* genes and *Rht8* would produce a too short phenotype. The selection away from *Rht8* to an allele that increases height could counteract the effects of *Ppd* genes and the GA-insensitive dwarfing genes and thus be of adaptive significance to the CIMMYT varieties.

The WMS 261-174-bp allele seems most widespread in the UK and much of north-western Europe. The common occurrence of this allele could be predicted by the selective advantage of photoperiod sensitivity in northern Europe and the linkage of *ppd1* to this allele in traditional varieties like 'Cappelle-Desprez' that show good adaptability to the area. A selective significance for WMS 261-174 bp can perhaps be drawn from modern photoperiod-insensitive French wheats. It is now accepted by French breeders that photoperiod insensitivity offers improved adaptability to varieties bred for growing in the South of France. Some of these varieties like 'Talent' and more recently 'Soissons' have also been a commercial success in the north of France and in the UK, where in recent years 'Soissons' has gained a 6% share of the wheat acreage. Whilst the older French-bred photoperiod-insensitive varieties like 'Etiole-de-Choisy' carried the *Ppd1*/*Rht8* linkage group from their Italian ancestors, more modern French varieties lack the WMS 261-192-bp microsatellite allele diagnostic for *Rht8* even though its presence can be shown in some parental varieties. The explanation for the selection of a recombined chromosome 2D linkage group could well be as for CIMMYT wheats, with modern photoperiod-insensitive French varieties like 'Courtot', 'Eureka', 'Soissons' and 'Victo' also carrying GA-insensitive dwarfing genes which together with *Rht8* and pleiotropic effects of *Ppd* genes could produce a phenotype too short.

Conclusions

The screening of varieties for allelic variants at the chromosome 2D locus of the wheat microsatellite WMS 261 demonstrated that the three main alleles tend to be distributed non-random in geographically divergent breeding programmes. The close linkage of WMS 261 to the dwarfing gene *Rht8* locus permits the use of the microsatellite as a marker for detection of allelic variants at the *Rht8* locus. As genetical and cytological analyses confirm the presence of *Rht8* in varieties, it has been possible to show that within the range of screened varieties where *Rht8* has been verified by genetical analysis breeding, selection has not broken the WMS 261/*Rht8* linkage. The analysis demonstrates *Rht8* to be present in southern European wheats, the standard 'Cappelle-Desprez' allele detected by WMS 261-174-bp in northern European wheats, and an allele that can be associated with height promotion occurs in CIMMYT varieties. Additional varietal screening is required to determine alleles present in other major wheat growing areas including Australia and China.

The detection of a height-promoting allele at the *Rht8* locus of CIMMYT wheats merits further consideration. The presence of such an allele could be deduced from experiments carried out by Hoogendoorn et al. (1988) who developed isogenic lines for GA-insensitive dwarfing genes and also a substitution line of the *Rht8*-carrying chromosome 2D of 'Mara' in a 'Maringa' background. 'Maringa' is an old Brazilian photoperiod-insensitive variety carrying the WMS 261-165-bp microsatellite allele derived via 'Frontana' from 'Mentana'. An 8-cm height reduction is associated with the 2D substitution line. As *Ppd1* is common to both parents this would have no effect on plant height. The 8-cm reduction in plant height must therefore be associated with a change from the height-promoting allele at the *rht*8 locus present in 'Maringa' to the height-reducing *Rht8* allele present in 'Mara'. No yield alteration was associated with changing alleles although the *Rht8* allele from 'Mara' could be associated with the production of more but smaller grains. A reduction in height of 8 cm would be in line with expectations for a comparison between the extreme height-promoting and height-reducing alleles at the *Rht8* locus. Previous comparisons have only been available to compare either the extreme dwarf *Rht8* allele or the tall WMS 261-165-bp allele against the medial 'Cappelle-Desprez' allele recognised by WMS 261-174-bp. The substitution line was not combined with *Rht-B1b* or *Rht-D1b* to determine interactions. Additional genetic stocks need to be developed to compare the extreme alleles in a 'Cappelle-Desprez' winter wheat background and to determine the effects of interaction between *Rht8* alleles and GA-insensitive dwarfing genes in winter and spring varietal backgrounds.

It will also be of interest to establish whether the small group of allelic variants of WMS 261 of more than 200 bp can be associated with additional height variants at the *Rht8* locus. The origins of the 6 varieties in this group is diverse with 4 being old varieties of uncertain parentage derived from Argentina.

The availability of a closely linked molecular marker for an agronomically important gene locus opens up many opportunities for further research into establishing the pleiotropic effects of differing alleles in characters such as yield and climatic adaptability. It also enables breeders to select appropriate alleles in their breeding programmes and should lead to swift improvements in varietal adaptability. Of particular significance here is the wealth of at least 6 allelic variants of the microsatellite that, if all prove to be linked to different variants at the *Rht8* locus, will offer enormous scope to breeders for optimising the gene to environment interaction.

Acknowledgements We thank Katja Wendehake for her excellent technical assistance. The microsatellite research was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG, Ro 1055/1-2). Regular contact between the German and UK research groups was made possible by grants awarded by the British - German Academic Research Collaboration Programme.

References

Borlaug NE (1968) Wheat breeding and its impact on world food supply. In: Finlay KW, Shepherd KW (eds) Proc 3rd Int Wheat Genet Symp, Austral Acad Sci, Canberra, pp 5–15

Börner A, Plaschke J, Korzun V, Worland AJ (1996) The relationship between the dwarfing genes of wheat and rye. Euphytica 89 : 69–75

Borojevic S, Potocanac J (1966) The development of the Yugoslav programme for creating high yielding wheat varieties. Davremena poljoprivreda 7–36 : 69–75

Dalrymple DG (1986) Development and spread of high yielding wheat varieties in developing countries. Bureau for Science and Technology, Washington, D.C.

Ford MA, Austin RB, Morgan CL (1985) Grain and straw yields of old and new wheat varieties. Plant Breeding Institute Annual Report 1984, Cambridge, UK, pp 106–107

Gale MD. Gregory RS (1977) A rapid method for early generation selection of dwarf genotypes in wheat. Euphytica 26 : 733–739

Gale MD, Marshall GA (1976) The chromosomal location of *Gai1* and *Rht1* genes for gibberellin insensitivity and semi-dwarfism in a derivative of Norin 10 wheat. Heredity 37 : 283–289

Gale MD, Youssefian S (1985) Dwarfing genes in wheat. In: Russell GE (ed) Progress in plant breeding. Butterworths and Co, London, pp 1–35

Gale MD, Law CN, Worland AJ (1975) The chromosomal location of a major dwarfing genes from Norin 10 in new British semi dwarf wheats. Heredity 35 : 417–421

Gale MD, Law, CN, Marshall, GA, Snape JW, Worland AJ (1982) The analysis and evaluation of semi-dwarfing genes in wheat, including a major height reducing gene in the variety 'Sava'. In: Semi-dwarf cereal mutants and their use in cross breeding. IAEA-TECDOC 268, Vienna, pp 7–23

Hoogendoorn J, Pfeiffer WH, Rajaram S, Gale MD (1988) Adaptive aspects of dwarfing genes in CIMMYT germplasm. In: Miller TE, Koebner RMD (eds) Proc. 7th Int Wheat Genet Symp. Bath press, Bath, pp 1093–1100

Korzun V, Röder MS, Ganal MW, Worland J (1998) Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Part I. Molecular mapping of the dwarfing gene *Rht8* on the short arm of chromosome 2D bread wheat (*Triticum aestivum* L.) Theor Appl Genet (in press)

Law CN, Worland AJ (1985) An effect of temperature on the fertility of wheats containing dwarfing genes *Rht1, Rht2* and *Rht3*. Annual Report Plant Breeding Institute 1984, Cambridge, UK, pp 69–71

Law CN, Snape JW, Worland AJ (1973) The genetical relationship between height and yield in wheat. Heredity 40 : 133–151

McIntosh RA, Hart GE, Gale MD (1995) Catalogue of gene symbols for wheat. In: Li ZS, Xin, ZY (eds) Proc. 8th Int. Wheat Genet Symp. Scientech Press, Beijing, pp 1333–1500

Mishra SK, Kushwaha CM (1995) Inheritance of *Rht8* dwarfing gene in wheat (*Triticum aestivum*). Indian J Genet 55 : 36–40

McVittie JA, Gale MD, Marshall GA, Westcott B (1978) The intrachromosomal mapping of the Norin 10 and Thom Thumb dwarfing gene. Heredity 40 : 67–70

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat varieties using microsatellite markers. Theor Appl Genet 91 : 1001–1007

Rajaram S, van Ginkel M (1996) Yield potential debate; germplasm vs. methodology or both in increasing yield potential in wheat: breaking the barriers. In: Reynolds, MP, Rajaram S, McNab A (eds) Increasing yield potential in wheat: Breaking the barrier. Proc. of workshop held in Ciudad Obregon, Sonora, Mexico. Published by CIMMYT, Mexico, pp 11–18

Snape JW, Law CN (1980) The detection of homologous chromosome variation in wheat using backcross-reciprocal monosomic lines. Heredity 45 : 187–200

Strampelli N (1932) Early ripening wheats and the advance of Italian wheat production. (in Italian.) Tipografia Failli, Rome

Sutka J, Kovacs G (1987) Chromosomal location of dwarfing gene *Rht12* in wheat. Euphytica 36 : 521–523

Worland AJ (1988) Catalogue of monosomic series. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp. Bath Press, Bath, UK, pp 1299–1403

Worland AJ (1996) The influence of flowering time genes on environmental adaptability in European wheats. Euphytica 89 : 49–57

Worland AJ, Law CN (1986) Genetic analysis of chromosome 2D of wheat. In:The location of genes affecting height, day length insensitivity and yellow rust resistance. Z. Pflanzenzüchtg 96 : 331–345

Worland AJ, Petrovic S (1986) The gibberellic acid insensitive dwarfing gene in the wheat variety Saitama 27. Euphytica 38 : 55–63

Worland AJ, Law CN, Shakoor A (1980) The genetical analysis of an induced height mutant in wheat. Heredity 45 : 61–71

Worland AJ, Petrovic S, Law CN (1988a) Genetic analysis of chromosome 2D of wheat II. The importance of this chromosome to Yugoslavian varieties. Plant Breed 100: 247–259

Worland AJ, Law, CN, Petrovic S (1988b) Pleiotropic effects of the chromosome 2D genes *Ppd1, Rht8* and *Yr16*. In: Miller TE, Koebner RMD (eds) Proc. 7th Int Wheat Genet Symp. Bath Press, Bath, UK, pp. 669–674

Worland AJ, Börner A, Petrovic S (1992) Genetics of final plant height in European winter wheats. EWAC Newsl 1992, pp 94–105

Worland J, Appendino, ML, Sayers EJ (1994) The distribution of genes influencing ecoclimatic adaptability in European winter wheat varieties. Euphytica 80 : 219–228

Worland J, Law CN, Petrovic S (1990) Height reducing genes and their importance to Yugoslavian winter wheat varieties. Savremena Poljo-privreda 38 : 245–258

Worland JM, Börner A, Korzun V, Li WM, Petrovic S, Sayers EJ (1997) The influence of photoperiod genes to the adaptability of European winter wheats. In: Braun HJ, Altay F, Kronstad WE, Beniwal SPS, McNab A (eds) Wheat: Prospects for Global Improvement. Proc 5th Int Wheat Conference, Ankara, Turkey. Kluwer Academic Publishers, pp 426–432