

Evidence of selective changes in winter wheat in middle-European environments reflected by allelic diversity at loci affecting plant height and photoperiodic response

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SUMMARY

Genes for adaptation to climatic conditions can have an impact on the expression of genes for agricultural productivity. This study tested the hypothesis that winter wheat cultivars registered in middle Europe (especially the Czech and Slovak Republics) during the period 1976–2009 were differentially adapted to different regions, reflecting selection for different allelic combinations. This was tested by analysing for the presence of alleles at the *Rht* and *Ppd* loci using molecular markers and gibberellic acids (GA) response tests. Four allelic variants (174, 192, 165 and 198-bp) were detected at the *Xgwm261* locus linked to *Rht8* on chromosome 2D. The 198-bp allele was rare, but present in some of the most widely grown cultivars. Of 85 cultivars grown in the area of Czech Republic, the 174-bp allele predominated in frequency and area (39 cultivars), often in combination with *Ppd-D1b* (30 out of 39 cultivars) and *Rht-D1b* (15 out of 30 cultivars). In neighbouring Slovakia, the 192-bp allele, generally associated with *Ppd-D1a*, was detected in 30 out of 40 cultivars; in 12 cultivars accompanied by a GA-insensitive allele on 4B chromosome (pedigree analyses indicated a high prevalence of the *Rht-B1d* allele). The 192-bp (*Rht8*)/*Ppd-D1a* linkage block was broken up in 7 out of 22 cultivars that carried the 192-bp allele in the ‘Czech collection’. Analysis of the effects of year of registration on allele frequency showed a decline in GA-insensitive cultivars released recently in both countries, and great changes in the frequency of the 2D alleles during the period 1981–2009 in the Czech Republic. The pedigrees of successful cultivars were examined to find probable sources of *Xgwm261* 192-bp, 174-bp and 165-bp alleles on 2D and *Rht* genes located on chromosomes 4B and 4D. These results will impact on breeding strategies and the exploitation of existing registered wheat cultivars in different regions and growing systems.

INTRODUCTION

The development of highly productive, short-stemmed wheat cultivars that are resistant to lodging has remained one of the main breeding objectives in most countries for many years. The introduction of the cultivars Norin 10, Saitama 27 and Akakomughi semi-dwarfing (*Rht*) genes in the first half of the

20th century led to significant worldwide increases in wheat yields (Gale & Yousefian 1985). The major *Rht* genes are classified into two groups according to their response to exogenous gibberellic acid (GA): GA-insensitive and GA-responsive (Gale & Gregory 1977). The Norin 10-derived GA-insensitive alleles *Rht-B1b* (previously termed *Rht1*) and *Rht-D1b* (*Rht2*), located on chromosomes 4B and 4D, respectively, cause a reduction in plant height of c. 15% and can increase yield by up to 20% (Worland & Law 1986). Their distribution is, however, restricted to

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geographic areas that are not subjected to heat stress during ear emergence, as this has been demonstrated to reduce plant fertility (Worland & Law 1986). Poor seedling emergence from depth because of shorter coleoptiles and lower adaptation, especially to dry environments, are other known problems connected with the presence of the *Rht-B1b* and *Rht-D1b* alleles (Trethowan *et al.* 2001). Landjeva *et al.* (2008) showed that seedling growth was significantly affected by the allelic status at the *Rht* loci, background genes and the water potential. In Southern Europe, it appeared advantageous to incorporate the Saitama 27-derived *Rht-B1d* allele, characterized by a weaker GA-insensitivity, less reduction in height and lower temperature sensitivity (Worland & Petrovic 1988; Ganeva *et al.* 2005).

The GA-responsive *Rht* genes, *Rht8* and *Rht9*, were introduced into Italian germplasm from the Japanese cultivar Akakomughi, and the 192-bp allele of the microsatellite marker, WMS 261, on chromosome 2D, was initially reported to be diagnostic for *Rht8* (Korzun *et al.* 1998). However, it was recently reported by Ellis *et al.* (2007) that the 192-bp allele at this locus is not always diagnostic for the height-reducing gene *Rht8*, stating that the presence of the *Xgwm261* 192-bp allele is only indicative of *Rht8* in wheat cultivars that have inherited this allele from Akakomughi or a Strampelli wheat ancestor. This allele now prevails in Southern and South-Eastern European genotypes and in Southern Ukraine, implying an adaptive significance of the 192-bp allele for these climatic conditions, evidently due to the beneficial effects on increased fertility (Chebotar *et al.* 2001; Worland *et al.* 2001). The *Xgwm261* 192-bp allele was also found to be prevalent in Chinese wheat cultivars (Liu *et al.* 2005). The *Xgwm261* locus is highly polymorphic with three main allelic variants, characteristic of cultivars Ciano 67 (165-bp), Cappelle-Desprez (174-bp) and Mara (192-bp). According to Korzun *et al.* (1998), it was possible to attribute a 70–80 mm reduction in plant height to the *Xgwm261* 192-bp allele compared to the *Xgwm261* 174-bp allele. A height reduction of *c.* 30 mm was detected between the *Xgwm261* 174-bp allele and the *Xgwm261* 165-bp allele. Screening of over 800 wheat cultivars from 20 countries (Worland *et al.* 2001) showed a high proportion (0.9) of these three alleles. Ten novel alleles at the *Xgwm261* locus were also detected, the most frequent being the 197-bp allele, present in 51 cultivars coming mainly from Austria, Holland and Switzerland. Four novel alleles (180, 198, 200 and 204-bp) were detected in the US and New Zealand wheat cultivars by Ahmad & Sorrells (2002).

Marker locus *Xgwm261* is in tight linkage on chromosome 2D with the semi-dwarfing gene *Rht8* and also with the photoperiod insensitive *Ppd-D1a* gene, which enabled simultaneous breeding for short stem and earliness connected with the transfer of a *Rht8/*

Ppd-D1a linkage block (Pestsova & Röder 2002). *Ppd-D1a* is extremely important in increasing the adaptability and yield in cultivars from Southern Europe. A selective advantage for the preservation of the linkage between *Ppd-D1a* and *Rht8* has been observed in Italian, Yugoslavian and Russian breeding programmes. In west-European cultivars and CIMMYT wheats, the absence of an adaptive advantage for the linkage block containing *Ppd-D1a/Rht8* led to the breakage of this linkage (Worland *et al.* 1998). The effects of the *Ppd-D1a* allele on yield potential appeared to be largely neutral in the conditions of the UK, but *Ppd-D1a* was associated with increased early biomass production and a more efficient production of grain dry matter (improved harvest index (HI)) (Foulkes *et al.* 2004). Addisu *et al.* (2010) recently demonstrated that the presence of *Ppd-D1a* in west-European regions may be also beneficial for early resource capture, which is highly desirable especially under organic agriculture (Wolfe *et al.* 2008).

It was clearly shown by Reynolds & Borlaug (2006) that crop improvement in general must embrace the problems associated with a highly heterogeneous and unpredictable environment, and it is highly desirable to achieve synergies between genetic improvement and innovative crop management practices. The best evidence of wide adaptation in variable environments was given by CIMMYT's shuttle breeding strategy, which resulted in the development of some cultivars (e.g. Siete Cerros, Anza, Sonalika and Seri 82) that were grown on several million ha (Braun *et al.* 1997). However, it is obvious that widely adapted genotypes are the best performers in only a narrow range of so-called mega-environments and usually not including those with severe stresses (Ceccarelli 1989). Therefore, the identification of the range of target environments in which the genotypes are likely to perform well after the application of certain farming practices will be a key objective (Wolfe *et al.* 2008). It can be expected that a high degree of buffering capacity is related to an appropriate plant growth type (i.e. the presence of certain *Rht* and *Ppd* alleles). An insight into the breeding history, reflecting the changes of breeding goals and requirements of agricultural practice (Phillips & Wolfe 2005; Peltonen-Sainio *et al.* 2008; Wolfe *et al.* 2008; Sener *et al.* 2009; Acreche & Slafer 2009; Šíp *et al.* 2009) could be helpful to find plant types suitable for certain growing conditions. Mohammadi *et al.* (2009) stated that a careful definition, with the participation of farmers, of target environments will ensure that the cultivars developed are adapted to the target conditions. Farmers and breeders are now faced with the effects of climatic changes, needs for organic production, lowering of inputs in agriculture and wider applications of reduced tillage systems, while keeping the productivity at the highest possible level. This study follows the dynamics of the distribution

of alleles affecting plant height and photoperiodic response in different European winter wheat cultivars that were registered in Middle Europe (especially the Czech and Slovak Republics) over the last 30 years in order to test the hypothesis that regional allelic combinations exist, and to find the appropriate combinations of alleles to use in certain growing regions and current agricultural systems.

MATERIALS AND METHODS

Plant material, data sources and description of growing conditions

This study focuses on 85 winter wheat cultivars that were registered in the Czech Republic during the period 1981–2009, and were still registered up to 1 January 2010. Their detailed characteristics are available on the website of the Central Institute for Supervising and Testing in Agriculture (CISTA), Brno, Czech Republic (<http://www.ukzuz.cz/Folders/2220-1-Plant+Varieties.aspx>). The collection of winter wheat cultivars grown in Slovakia included 40 cultivars registered by the Slovak Central Controlling and Testing Institute in Agriculture (CCTIA) since 1976. All cultivars were the highest yielding on the date of registration. The Czech collection includes cultivars bred in eight European countries (31 in the Czech Republic, 31 in Germany, eight in Slovakia, eight in the Netherlands, three in France, two in Austria, one in Denmark and one in the UK). The Slovak collection contained only cultivars of Slovak origin, because they have dominated cultivation up to the present time. Breeders' S1 seed (Pre-Basic) was used for the analyses of cultivars registered in the Czech and Slovak Republics.

Despite being close geographically, the main wheat-growing areas of the Czech Republic and the Slovak Republic represent different and variable environmental conditions in the middle of Europe. Thus, a different germplasm has been exploited in wheat breeding in these regions. A characteristic feature of Czech wheat breeding and cultivation is the wide use of germplasm from the UK and Western Europe, and previously, to a large extent also from the Ukraine and Russia, as well as the registration of cultivars that come from these regions. In Slovakia, Eastern and South European, germplasm has a higher importance, allowing for an earlier genotype.

The Czech Republic lies in a temperate climatic zone with pleasantly mild summers. The temperature difference between summers and winters is relatively high due to its landlocked geographical position. Alternation between hard and relatively mild winters is quite typical for this region. The climate in Slovakia can be considered similar, but the tendency to a continental type of climate is more marked. The main difference concerns the relatively low proportion of

agricultural land situated in maize-growing regions in the Czech Republic (0.07), while it is higher in Slovakia (0.37). The proportions of agricultural land used for cereal, sugar beet, potato and upland fodder crops in the growing regions of the Czech Republic are 0.41, 0.24, 0.18 and 0.10, respectively. The growing of cereals in Slovakia is highly concentrated in the maize and sugar beet regions, occupying 0.59 of the agricultural land in total. The remaining 0.41 of land is situated in the potato and upland regions, where cereal growing is very limited.

Brief characteristics of the main regions used for growing wheat

The maize-growing region typically is at altitudes up to 250 m asl, has an annual average precipitation of 500–600 mm, an annual mean air temperature of 9–10 °C and 0.30–0.50 of the year lacks rainfall. The characteristics of the sugar beet growing region are altitude: 250–350 m asl, annual average precipitation: 500–650 mm, annual mean air temperature: 8–9 °C, proportion of the year that lacks rainfall: 0.10–0.60. Those of the cereal growing region (Czech Republic) are altitude: 300–600 m asl, annual average precipitation: 550–700 mm, annual mean air temperature: 5–8.5 °C, proportion of the year that lacks rainfall: 0.05–0.40 and those of the potato growing region are altitude: 400–650 m asl, annual average precipitation: 550–900 mm, annual mean air temperature: 5–8 °C and proportion of the year that lacks rainfall: 0.05–0.30.

The proportion of agricultural land used for growing wheat fluctuates according to the season, but it is, in general, higher in the sugar beet-, maize- and cereal-growing regions (0.29–0.36) than in the potato-growing region (0.23–0.25). Yield potential of the registered wheat cultivars, estimated from the Official Trials of CISTA and CCTIA (2005–09) was 9.32 t/ha, and was highest in the sugar beet region (yield was 10.7% higher than average), whereas it was 3–5% below average in the remaining growing regions.

Characterization of Rht and Ppd genotypes

Responses to exogenously applied GA were determined using the method of Gale & Gregory (1977). To distinguish between GA-responsive and GA-insensitive genotypes, the method consisted of a 14-day treatment with 50 ppm GA₃. Measurements of shoot length were taken 10 and 14 days after GA treatment and in control (untreated) plants. A genotype was considered to be GA-sensitive when the increase in shoot length between the GA-treated and control plants was statistically significant.

Genomic DNA was extracted from individual plants using commercial DNeasy Plant Mini Kits (Qiagen)

following manufacturer's instructions. For detection of both the *Rht-B1* and *Rht-D1* genes (except for the *Rht-D1a* allele), located on wheat chromosomes 4B and 4D, respectively, polymerase chain reaction (PCR) conditions and primers were as published by Ellis *et al.* (2002). To detect the *Rht-D1a* allele, reaction conditions were: initial denaturation at 94 °C for 3 min, followed by 45 cycles (94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min) and finished at 72 °C for 10 min. The PCR products were separated on agarose gels and visualized using ethidium bromide.

Probable allelic variation associated with the *Rht8* locus on chromosome 2D was assessed using the microsatellite marker WMS 261 (Korzun *et al.* 1998) with slight changes in reaction mixture and conditions. The reaction mixture (total volume of 15 µl) contained: 1.5 µl of 2 mM Deoxyribonucleoside triphosphate (dNTP), 1.5 µl of both 2 µM primers, 1.5 µl of buffer, 0.07 of *Taq* polymerase and 5 µl of template DNA (20 µg. µl⁻¹). PCR conditions were: 94 °C 1 min (94 °C for 1 min, 60 °C 1 min, 72 °C 1 min) × 30 cycles and final step at 72 °C for 4 min.

PCR products were then separated on 5% polyacrylamide gel (1 litre of stock solution contained 125 ml of 40% acrylamide/bis-acrylamide solution [19:1 ratio], Sigma Aldrich product no. A9926) and visualized by silver staining (Bassam *et al.* 1991). The *Xgwm261* allele size was determined by juxtaposition with control cultivars Bezostaya 1 for the 192-bp allele, Mironovskaya 808 for the 174-bp allele, Kosutka for the 165-bp allele. Varieties carrying alleles larger than 192-bp were screened using the ABI PRISM[®] 310 Genetic Analyser.

Allelic variants at the *Ppd-D1* locus on chromosome 2D were also examined. Primers and PCR conditions were used as described in detail by Beales *et al.* (2007). A 288-bp product was indicative of the presence of the photoperiod-insensitive *Ppd-D1a* allele and a 414-bp product of the presence of the photoperiod-sensitive *Ppd-D1b* allele.

Pedigree analyses

Pedigrees of the registered cultivars were examined using the European Wheat Database (EWDB) held at the Crop Research Institute, Prague (<http://www.ecpgr.cgiar.org/databases/crops/wheat.htm>, verified 17 August 2010). The data on the presence of the *Rht* and *Ppd* alleles examined in different cultivars included in the pedigree diagrams were mainly obtained from the literature (Worland *et al.* 1998; Chebotar *et al.* 2001; Ganeva *et al.* 2005). The cultivars that still required examination were analysed in the Crop Research Institute, Prague and the Research Institute of Plant Production Piešťany, if the seed was available in the Gene Bank Departments of these institutes.

RESULTS

Gibberellin tests and molecular genotyping for GA-insensitive Rht genes

The results of the gibberellin tests corresponded with the molecular analyses. Screening showed that 54 out of the 85 cultivars registered in the Czech Republic (0.64) were GA responsive and 31 (0.37) were GA insensitive. Among the cultivars registered in the Slovak Republic, the proportions were very similar (0.65 of GA-sensitive cultivars and 0.35 of GA-insensitive cultivars). However, as demonstrated in Table 1, the proportion of *Rht-B1b/d* and *Rht-D1b* genotypes differed greatly between the two countries. While in Slovakia only the presence of *Rht-B1b* or *d* was detected by the diagnostic molecular marker, in the Czech Republic, the presence of *Rht-D1b* was dominant (0.84 of GA-insensitive cultivars). From pedigree analyses, the predominant allele at *Rht-B1* should be *Rht-B1d*, because 12 out of 16 GA-insensitive winter wheat cultivars (cvvars Agra, Astella, Barbara, Baletka, Danubia, Istra, Regia, Solara, Solaris, Solida, Velta and Venistar) have the cultivar Produttore (descendant of Saitama 27) in their pedigree. In the cultivar Viador, the source of the *Rht* genes is not clear from pedigree analysis. In Ilona and Iris, the source of the *Rht-B1b* allele could be Siete Cerros. The widely grown Czech cultivar Rheia, which is high yielding particularly in the maize-growing region, has in its pedigree the cultivar Vlada, in which the phenotyping results showed a significantly longer shoot length than in Siete Cerros, which may indicate the presence of weaker insensitivity characteristic of *Rht-B1d*.

It is clear from Table 2 that *Rht-D1b* cultivars appeared in Czech agriculture at a higher frequency after 1995, and for the whole period 1981–2009, the presence of *Rht-B1d* or *b* was the exception. In Slovakia, this allele eventually dominated. The occurrence of *Rht-B1d* or *b* genotypes has dropped from being common during the period 1976–94 to a low presence currently.

Distribution of allelic variants at Xgwm261 and Ppd-D1

Among the cultivars examined at the *Xgwm261* molecular marker locus associated with *Rht8* height allelic variants, four different alleles were detected in both countries: 165, 174, 192 and 198-bp. However, the distribution of these alleles was different in the neighbouring countries. As shown in Tables 1 and 2, the 174-bp allele was the most frequent from 1995 in the Czech Republic, detected in 39 out of 85 cultivars (0.46). In total, 20 cultivars (0.24) were found to carry the 165-bp allele, 22 cultivars (0.26) the 192-bp allele and only four cultivars (0.05) the 198-bp allele. Relative to the year of registration, the proportion of 165-bp allele increased from 0.07 to 0.38. Before 1995,

Table 1. Distribution of winter wheat cultivars grown in the Czech and Slovak Republics according to their reaction to GA (I: presence of genes Rht-B1b or d and Rht-D1b), alleles at the Xgwm261 locus and alleles at the Ppd-D1 locus

Response to GA*; <i>Rht</i> allele; <i>Xgwm261</i> allele; <i>Ppd-D1</i> allele	No.	Proportion	Cultivars†						
Czech Republic									
R; <i>Rht8</i> (192-bp); <i>Ppd-D1a</i>	10	0.12	Banquet (CZ)	Blava (SK)	Hana (CZ)	Livia (SK)	Mona (CZ)	Niagara (CZ)	Samanta (CZ)
R; <i>Rht8</i> (192-bp); <i>Ppd-D1b</i>	6	0.07	Saskia (CZ)	Svitava (CZ)	Viginta (SK)				
R; 165-bp; <i>Ppd-D1a</i>	1	0.01	Asta (CZ)	Nela (CZ)	Regina (CZ)	Samara (CZ)	Simila (CZ)	Sultan (CZ)	
R; 165-bp; <i>Ppd-D1b</i>	10	0.12	Kosutka (SK)	Brilliant (DE)	Buteo (DE)	Dromos (DE)	Ebi (DE)	Eurofit (AT)	Kerubino (DE)
R; 174-bp; <i>Ppd-D1a</i>	8	0.09	Magister (DE)	Manager (DE)	Raduza (CZ)				
R; 174-bp; <i>Ppd-D1b</i>	15	0.18	Alana (CZ)	Alibaba (DE)	Alka (CZ)	Bohemia (CZ)	Darwin (DE)	Drifter (DE)	Hedvika (NL)
R; 198-bp; <i>Ppd-D1a</i>	2	0.02	Ilias (NL)						
R; 198-bp; <i>Ppd-D1b</i>	2	0.02	Apache (FR)	Bakfis (CZ)	Barryton (DE)	Bill (DE)	Complet (DE)	Estica (NL)	Etela (CZ)
I; <i>Rht-B1d</i> ; 174-bp; <i>Ppd-D1b</i>	1	0.01	Federer (CZ)	Ludwig (AT)	Mulan (DE)	Orlando (DK)	Sakura (CZ)	Semper (NL)	Siria (CZ)
I; <i>Rht-B1d</i> or b; 192-bp (<i>Rht8</i>); <i>Ppd-D1a</i>	5	0.06	Tower (NL)						
I; <i>Rht-D1b</i> ; 165-bp; <i>Ppd-D1b</i>	7	0.08	Meritto (CZ)	Sida (CZ)					
I; <i>Rht-D1b</i> ; 165-bp; <i>Ppd-D1a</i>	2	0.02	Akteur (DE)	Sulamit (CZ)					
I; <i>Rht-D1b</i> ; 174-bp; <i>Ppd-D1b</i>	14	0.17	Rheia (CZ)						
I; <i>Rht-D1b</i> ; 174-bp; <i>Ppd-D1a</i>	1	0.01	Astellia (SK)	Baletka (CZ)	Ilona (SK)	Solara (SK)	Rexia (syn. Regia) (SK)		
I; <i>Rht-D1b</i> ; 192-bp; <i>Ppd-D1b</i>	1	0.01					Record (DE)	Trend (DE)	Vlasta (CZ)
			Contra (DE)	Florett (DE)	Globus (DE)	Rapsodia (DE)			
			Caphorn (FR)	Mladka (CZ)					
			Athlet (DE)	Barroko (CZ)	Biscay (DE)	Clarus (DE)	Clever (DE)	Corsaire (FR)	Heroldo (DE)
			Karolinum (NL)	Kodex (DE)	Rialto (UK)	Ritmo (NL)	Sepstra (DE)	Versailles (NL)	Windsor (DE)
			Sarka (CZ)						
			Cubus (DE)						
Slovak Republic									
R; 165-bp; <i>Ppd-D1a</i>	2	0.05	Kosutka	Vanda					
R; 165-bp; <i>Ppd-D1b</i>	2	0.05	Malvina	Malyska					
R; 174-bp; <i>Ppd-D1a</i>	2	0.05	Pavla	Torysa					
R; 192-bp (<i>Rht8</i>); <i>Ppd-D1a</i>	18	0.45	Amika	Arida	Armelis	Axis	Blava	Bonita	Eva
			Ignis	Klea	Livia	Markola	Petrana	Rada	Sana
			Stanislava	Veldava	Viginta	Zerda			
R; 198-bp; <i>Ppd-D1b</i>	2	0.05	Roxana	Verita					
I; <i>Rht-B1d</i> ; 198-bp; <i>Ppd-D1a</i>	1	0.03	Viador						
I; <i>Rht-B1d</i> ; 165-bp; <i>Ppd-D1a</i>	2	0.05	Istra	Solaris					
I; <i>Rht-B1d</i> or b; 192-bp (<i>Rht8</i>); <i>Ppd-D1a</i>	11	0.28	Agra	Astellia	Barbara	Danubia	Ilona	Iris	Regia
			Solara	Solida	Velta	Venistar			

* R, responsive to GA; I, insensitive to GA.

† In brackets – country of origin based on EVIGEZ database (<http://genbank.vurv.cz/genetic/resources/>); in Slovak Republic all cultivars SK.

Table 2. Effect of registration year on the presence of alleles at the *Xgwm261* locus, GA-insensitive alleles *Rht-B1b* or *d* and *Rht-D1b* and alleles at the *Ppd-D1* locus in the winter wheat cultivars grown in the Czech and Slovak Republics

Gene/allele	Czech Republic						Slovak Republic							
	1981–94 (n = 15)		1995–99 (n = 21)		2000–04 (n = 25)		2005–09 (n = 24)		1976–94 (n = 15)		1995–2001 (n = 13)		2002–07 (n = 12)	
	No	Proportion	No	Proportion	No	Proportion	No	Proportion	No	Proportion	No	Proportion	No	Proportion
165-bp	1	0.07	4	0.19	6	0.24	9	0.38	3	0.20	3	0.23	0	0.00
174-bp	3	0.20	11	0.52	13	0.52	12	0.50	1	0.07	0	0.00	1	0.08
192-bp	10	0.67	6	0.29	3	0.12	3	0.12	10	0.67	10	0.77	9	0.75
198-bp	1	0.07	0	0.00	3	0.12	0	0.00	1	0.07	0	0.00	2	0.17
<i>Rht-B1d</i> or <i>b</i>	1	0.07	2	0.09	1	0.04	1	0.04	8	0.53	5	0.38	2	0.17
<i>Rht-D1b</i>	0	0.00	10	0.48	10	0.40	5	0.21	0	0.00	0	0.00	0	0.00
S*	14	0.93	9	0.43	14	0.56	18	0.75	7	0.47	8	0.62	10	0.83
<i>Ppd-D1a</i>	11	0.73	6	0.29	10	0.40	2	0.08	14	0.93	11	0.85	11	0.92
<i>Ppd-D1b</i>	4	0.27	15	0.71	15	0.60	22	0.92	1	0.07	2	0.15	1	0.08

* S = sensitive to GA.

10 (0.67) cultivars carried the *Xgwm261* 192-bp allele that could be associated with the *Rht8* dwarfing gene, but only three cultivars (0.12) were detected as carrying this allele in the registration period 2005–9. The *Xgwm261* 192-bp allele was common in cultivars registered in the Slovak Republic (detected in 30 out of 40 cultivars, 0.75) and its occurrence was independent of the year of registration. The remaining alleles occurred at a lower proportion, which ranged from 0.13 (165-bp allele), 0.08 (198-bp allele) to 0.05 (174-bp allele).

In the Slovak Republic, 36 out of 40 cultivars (0.90) were found to carry the allele *Ppd-D1a* for insensitivity to photoperiod (Table 1). The linkage between the alleles 192-bp (indicative of *Rht8*) and *Ppd-D1a* has not been broken in any cultivar. The proportion of *Ppd-D1a* in cultivars registered in the Czech Republic was 0.34 (in 29 out of 85 cultivars), and 15 out of 29 cultivars (0.52) have this allele associated with the 192-bp allele.

The most frequent allelic combinations among GA responsive genotypes were *Xgwm261* 174-bp/*Ppd-D1b* (15 cultivars), 165-bp/*Ppd-D1b* (10 cultivars) and *Xgwm261* 192-bp/*Ppd-D1a* (10, mostly older, cultivars) in the Czech Republic. The GA-insensitive gene *Rht-D1b* is often combined with alleles 174-bp and *Ppd-D1b* (14 cultivars, Table 1). In the Slovak Republic, the following allelic combinations were the most frequent: *Xgwm261* 192-bp/*Ppd-D1a* (18 cultivars) and *Rht-B1b* or *d*/*Xgwm261* 192-bp (*Rht8*)/*Ppd-D1a* (12 cultivars).

Sources of different allelic variants at the *Xgwm261*, *Rht* and *Ppd-D1* loci in cultivars adapted to target environments

The classification of winter wheat cultivars grown in Middle Europe by the presence of the GA-insensitive dwarfing genes (*Rht-B1b*, *Rht-B1d* and *Rht-D1b*) and alleles at the *Xgwm261* and *Ppd-D1* loci is given in Table 1. Successful cultivars were traced back to find probable sources of *Xgwm261* 192-bp, 174-bp and 165-bp alleles on 2D and *Rht* genes on chromosomes 4B and 4D (Figs 1–4).

It is obvious from Fig. 1 that the main ‘domestic’ sources of the *Xgwm261* 192-bp allele, which may be diagnostic for *Rht8*, were cvs Hana, Viginta and Slavia, registered in the Czech Republic in 1985, 1984 and 1976, respectively. All these cultivars occupied large areas and were widely exploited in the Czech and Slovak wheat-breeding programmes. The older Russian cultivars (especially Mironovskaya 808) evidently contributed the high levels of winter-hardiness and bread-making quality in these cultivars. The Ukrainian cultivars (Bezostaya 1 and Ilichevka) were evidently donors of the 192-bp *Rht8*-associated allele for all these cultivars (directly for cvs Viginta and Slavia and probably through the Serbian line

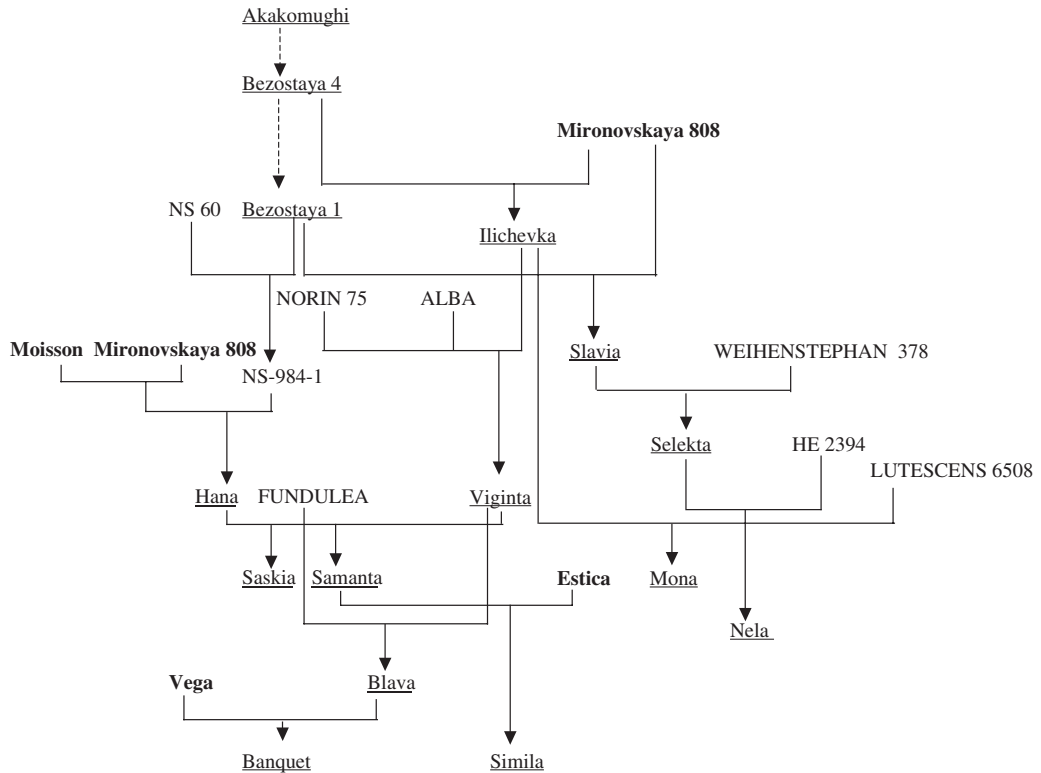


Fig. 1. Pedigrees of Czech winter wheat cultivars carrying *Xgwm261* 192-bp allele (lower case, underlined). Bold letters: allele 174-bp. Capital letters: not tested. Dashed lines connecting the cultivars denote probable gene flow from well-known gene sources.

NS-984-1 for Hana). It is generally known that the cultivar Hana, which also possesses *Ppd-D1a*, was widely and successfully used as a donor of improved bread-making quality and earliness. Figure 2 also shows that the second important transfer of the Akakomughi *Rht8* gene (see pedigree of the Slovak cultivar Ilona) could also have happened through the Mexican cultivar Siete Cerros, in which, however, the origin of the *Xgwm261* 192-bp allele could not be ascertained by pedigree analysis. The alternative route for this allele to Ilona can be via the Russian cultivar Kavkaz (derived from Bezostaya 1). It is evident from Table 1 that in the Czech cultivars Asta, Nela, Samara, Simila and Sultan, the 192-bp (*Rht8*)/*Ppd-D1a* linkage block was broken, as in the current GA-insensitive German cultivar Cubus, and the famous older cultivar Regina, which was widely grown particularly in the potato growing region (the source of *Rht8* gene in cvs Sultan, Cubus and Regina) was not found. As shown in Table 2, the presence of the *Xgwm261* 192-bp (*Rht8*) in modern cultivars grown in the Czech Republic is very rare (since 2005, it is only found in cvs Simila, Baletka and Sultan), but it is

still frequent in Slovakia, where the 192-bp (*Rht8*)/*Ppd-D1a* linkage has not yet been broken. In Slovak cultivars, the 192-bp (*Rht8*) genotype was often combined with *Rht-B1d* or *Rht-B1b* (possible pathways can be found in Fig. 2). The probable donor of the *Rht-B1d* allele from cvar Saitama 27 to modern Slovak cultivars could be the line SO 985, derived from the cross between the cultivars Nebojska and Produktore. The presence of Norin 10 dwarfing genes has not been detected in any *Rht8* cultivar bred in the Czech Republic.

As seen in Tables 2 and 3, the 174-bp allele of the *Xgwm261* locus became highly prevalent in the Czech wheat-growing regions after 1995, and *Ppd-D1b* at the expense of *Ppd-D1a*. This can be connected to the introduction of UK and West European high-yielding cultivars that also often carry *Rht-D1b*. As seen in Fig. 3, cvar Capelle-Desprez probably was the source of the 174-bp allele for the modern Czech cultivars Sakura and Bohemia, through cvar Maris Huntsman and subsequently cvs Virtue, Mercia and Estica. Cultivar Maris Huntsman also can be considered as the probable source of the 174-bp allele for the

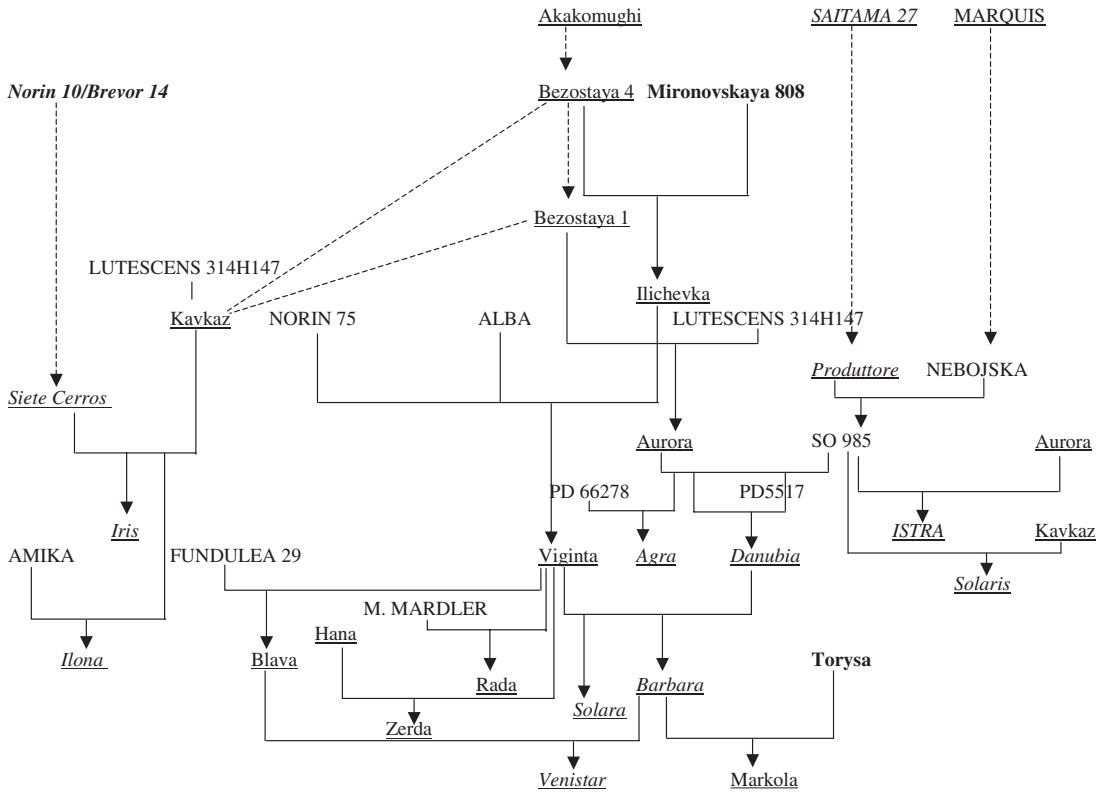


Fig. 2. Pedigrees of Slovak winter wheat cultivars carrying *Xgwm192* 192-bp allele (lower case, underlined). Bold letters, lower case: allele 174-bp. Italics: GA-insensitive (*Rht-B1b* or *d*). Capital letters: not tested. Dashed lines connecting the cultivars denote probable gene flow from well-known gene sources.

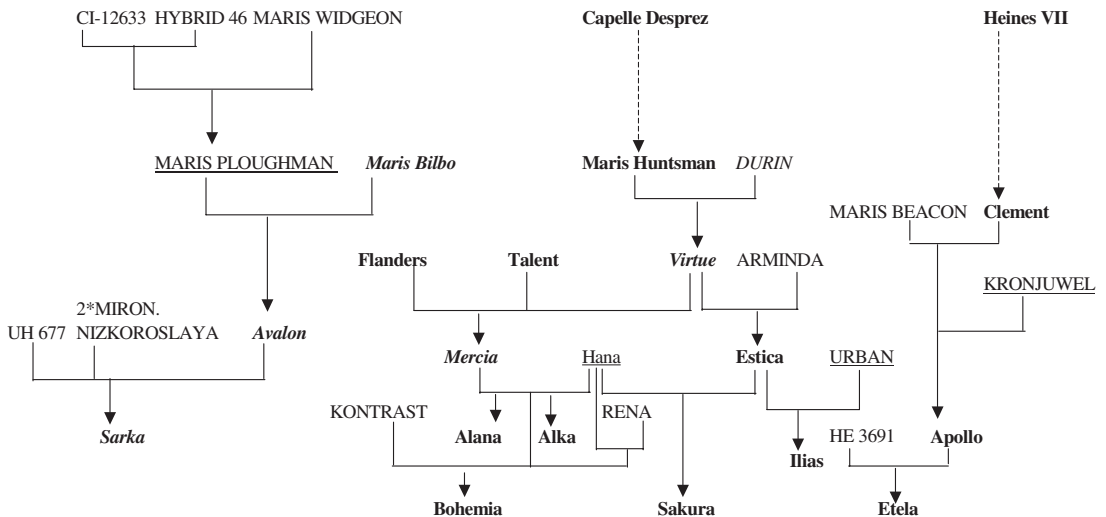


Fig. 3. Pedigrees of Czech winter wheat cultivars carrying *Xgwm261* 174-bp allele (bold lower case). Italics: GA-insensitive (*Rht-D1d*). Capital letters, underlined: 165-bp allele. Capital letters: not tested. Dashed lines connecting the cultivars denote probable gene flow from well-known gene sources.

Table 3. Distribution of *Rht* and *Ppd* alleles among commercial winter wheats in the Czech and Slovak Republics; area of seed multiplication in 2007

	Czech Republic		Slovak Republic	
	No. of cultivars	Sown area (proportion)*	No. of cultivars	Sown area (proportion)†
<i>Xgwm261</i> allele				
165-bp	9	0.19	14	0.08
174-bp	16	0.41	23	0.15
192-bp	3	0.07	32	0.34
198-bp	3	0.19	6	0.08
<i>Rht-B1b</i> or <i>d</i> , <i>Rht-D1b</i>	11	0.25	27	0.10
<i>Rht-B1a</i> , <i>Rht-D1a</i>	20	0.61	48	0.55
<i>Ppd-D1a</i>	9	0.23	48	0.48
<i>Ppd-D1b</i>	22	0.62	27	0.17
Sum	31	0.85	75	0.65

* Included only registered cultivars with sown area >0.01.

† 0.35 of area is occupied by Slovak and foreign cultivars in which the allelic composition was not yet identified.

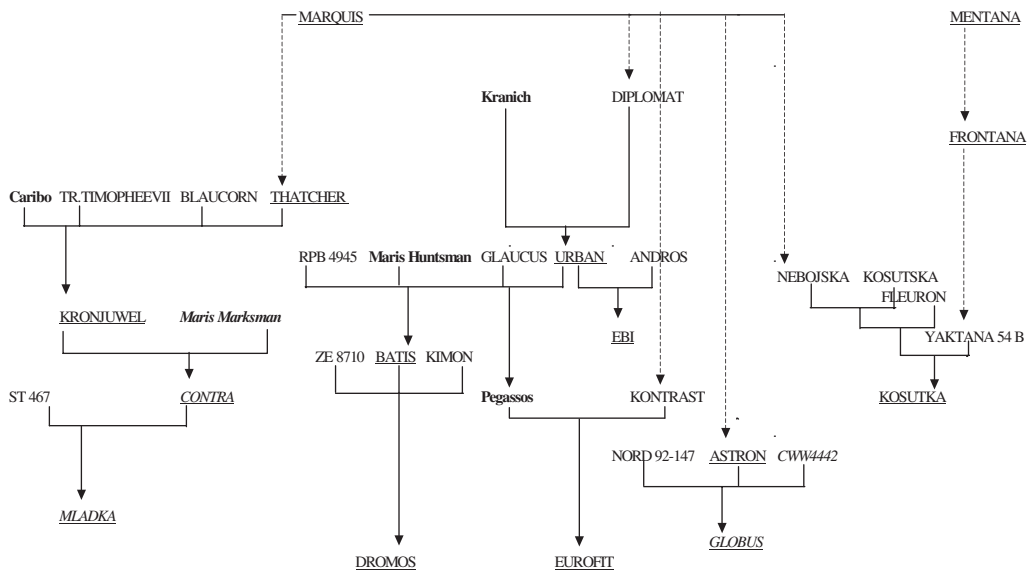


Fig. 4. Pedigrees of Czech and adapted foreign winter wheat cultivars carrying *Xgwm261* 165-bp allele (capitals, underlined). Italics: GA-insensitive (*Rht-D1d*). Lower case, bold: 174-bp allele. Capital letters: not tested. Dashed lines connecting the cultivars denote probable gene flow from well-known gene sources.

successful cultivars of foreign origin such as Darwin, Drifter, Complet, Biscay, Mulan and Ritmo (see <http://genbank.vurv.cz/ewdb>, verified 17 August 2010). The cultivars Heines VII, Clement and Apollo could be sources of the 174-bp allele for the very high-yielding modern cultivar Etela (bred by the Limagrains Central Europe Cereals, Ltd, Plant Select, Hrubčice, CZ). The proportion of *Rht-D1b* and *Rht-D1a* cultivars carrying the 174-bp and *Ppd-D1b* alleles

was almost identical (0.17 and 0.18). However, most of the registered GA-insensitive cultivars are foreign, mainly west European cultivars. The short, early and lodging-resistant Czech cultivar Sarka appears to be highly adapted to these climatic conditions and carries *Rht-D1b* (from the British cultivar Avalon) in combination with *Ppd-D1a*. Although sources of *Rht-D1b* (British cultivars Virtue and Mercia) are involved in the pedigrees of modern Czech cultivars, the selection

for greater plant height, earliness, high grain quality and winter hardiness probably resulted in the retention of non-*Rht-D1b* genotypes. To obtain the necessary earliness, the presence of *Ppd-D1a* was evidently beneficial in nine cultivars, and among them Alana, Bohemia and the Dutch cultivar Ilias were widely exploited in agricultural practice.

It is obvious from Fig. 4 that the key source of the *Xgwm261* 165-bp allele appears to be the Canadian spring wheat cultivar Marquis, released in 1911 and widely exploited by spring wheat breeders in the USA and Canada. The route for the spread of the 165-bp allele to and around Europe was evidently through the North American cultivars Thatcher, Marquillo and probably also Reward (parent of the Slovak landrace Nebojska). The presence of this allele can be traced back to Marquis and alternatively also to the Italian cultivar Mentana, selected by Strampelli (Strampelli 1932). From cvar Mentana this allele was transferred to the Brazilian cultivar Frontana and to semi-dwarf CIMMYT cultivars. However, no clear route for the 165-bp allele from the well-known Mexican sources such as cvar Ciano 67 was not found in any 165-bp genotype examined; however, the UK cultivars Rapsodia and Caphorn have Ciano 67 in their pedigree. There are no data available on the presence of this allele in the Mexican cultivar Yaktana 64, derived from Frontana, which is a parent of the old awned Slovak cultivar Kosutka, widely grown particularly in warm, dry regions. Among the GA-responsive cultivars, the German cultivars Ebi and Batis were widely exploited in agriculture. Probable donors of the 165-bp allele in this genotype group were the German cultivars Urban and Diplomat that have cvar Marquis in their pedigrees. Another important branch is GA-insensitive cultivars, in which sources of *Rht-D1b* were the British cultivars Maris Marksman, Norman, Haven, Brimstone, PBIS 95-82 and CWW 4442. Many of these cultivars were also successful in agriculture, mainly the Czech cultivar Mladka that showed broad adaptability (very high yields in all regions in non-stressed conditions of the years 2000, 2001 and 2002), the German cultivars Rapsodia, Florett and Globus, and the Czech cultivar Vlasta suitable for growing in the potato region. The combination of the 'Mexican' 165-bp allele with *Ppd-D1a* occurred in three cultivars and the numbers of *Rht-D1a* and *Rht-D1b* genotypes carrying *Ppd-1Db* were 10 and 7, respectively (Table 1).

DISCUSSION

These results suggest that selection and adaptation to particular environments in Middle Europe are reflected by the presence of particular allelic combinations of *Rht* and flowering time loci. In this adaptation spectrum, there are common and also distinct features relating to the breeding history and the evolution of foreign cultivars particularly in two

neighbouring countries, the Czech Republic and Slovakia. These correspond to the climatic diversity of the middle European regions (the predominant wheat growing area and main breeding centre is situated in the maize region in Slovakia, while in the Czech Republic it is found in cereal, sugar beet and maize regions), and to a certain degree with specific historical developments. The breeding objectives did not differ very much, but the significance of the 'border position' of the Czech Republic between the continental and maritime types of climate evidently played an important role. The breeders soon became aware of the usefulness of combining properties of west and east European germplasm in order to obtain broader adaptation and good bread-making quality (Šíp *et al.* 1991). Pedigree analyses (Figs 1–4) also show that in the Czech Republic the cultivated modern winter wheat cultivars are derivatives of geographically diverse germplasm, and also that the allelic combinations are more variable than in the cultivars grown in the Slovak Republic. The genetic diversity studies of Martynov *et al.* (1997) showed that the majority of Czech and Slovak cultivars released in the period 1954–94 were related because they had common ancestors (35 of 62 cultivars analysed were found to be descendants of Canadian cultivars Marquis or Garnet). Martynov *et al.* (1997) also documented that in the two decades before 1994, the ratio of 'local germplasm' in the pedigrees of 'modern' cultivars has decreased substantially, and that the Ukrainian cultivars Mironovskaya 808 and Bezostaya 1 had the largest contribution (0.80). Cultivars containing *Xgwm261* 192-bp (*Rht8*) and *Ppd-D1a* came to dominate in both countries (in Slovakia often in combination with *Rht-B1d* or *Rht-B1b*). However, while these genotypes are still highly prevalent in Slovakia, a decline of cultivars containing *Xgwm261* 192-bp (*Rht8*) and *Ppd-D1a* has occurred since 1995 in the Czech Republic (Table 3), and, moreover, selection performed in relatively colder districts has led to genotypes that break the 192-bp (*Rht8*)/*Ppd-D1a* linkage block, unlike in Slovakia. However, it should be noted that the breakage of this linkage need not necessarily concern the association between *Rht8* and *Ppd-D1a*, because, as documented by Ellis *et al.* (2007), we cannot preclude a lack of association of the 192-bp allele with *Rht8*. The wide use of foreign winter wheat cultivars, especially from the UK, Germany, France and the Netherlands, has contributed to an increase in genetic diversity in winter wheat cultivars grown in the Czech Republic, as documented by Stehno *et al.* (2003). Recently, the frequent registration of foreign cultivars is also a characteristic of the Slovak Republic system. Among winter wheats registered in Slovakia in 2007, 0.09 of the cultivars are of Czech or west European origin (registered also in the Czech Republic), although not yet cultivated on a large area. Efforts to broaden the genetic base and increase

the availability of different growth types are now characteristics of both countries.

In Slovakia, there was a very low frequency of the *Xgwm261* 174-bp allele, the most frequent in the Czech Republic. While the routes of the *Xgwm261* 192-bp and 174-bp alleles are more easily identifiable, an inspection into the sources of the third important allele, 165-bp, is more complicated and often not clear. These investigations indicate that the main, obvious, donors of this allele were the Canadian cultivar Marquis and the Brazilian cultivar Frontana (derived from the Strampelli cultivar Mentana), mainly via the German cultivars Urban and Kronjuwel (derived from the US cultivar Thatcher). No clear paths, however, lead to the Mexican spring wheat cultivars, except for the Slovak cultivar Iris, coming from the cross between cvs Siete Cerros and Kavkaz. The GA-insensitive dwarfing gene *Rht-D1b* was not introduced to the UK and the other western European cultivars from CIMMYT germplasm, but via Chile. The possible bridge could be the US cultivar Vogel-8058. Alternatively, as documented by Šíp *et al.* (1995), the CIMMYT cultivars were widely exploited in Czech breeding programmes for the development of spring wheat cultivars carrying *Rht-B1b* gene. In winter wheat, there is little evidence of wide exploitation of the Mexican sources of either Norin 10 genes or the 165-bp allele of the *Xgwm261* locus.

There is a striking difference between these two countries in the use of GA-insensitive *Rht* genes located on chromosomes 4B and 4D. Only two cultivars bred in the Czech Republic (Rheia and Baletka) appear to carry the *Rht-B1d* Saitama 27 allele, and both cultivars were high yielding, particularly in the maize-growing region (Bobková *et al.* 2002; Laml & Pánek 2008). In the warmer conditions of neighbouring Slovakia, the *Rht-D1b* allele was not detected and the majority of GA-insensitive genotypes probably carry *Rht-B1d*. These findings support the usefulness of exploiting the *Rht-B1d* allele in the conditions of Southern Europe, often in combination with *Rht8* and *Ppd-D1a* (Worland & Petrovic 1988; Worland *et al.* 2001). It has been stated that the *Rht-D1b* genotypes do not perform well in warmer areas, which is consistent with these and other results (Šíp *et al.* 2010). The effects of the Norin 10 dwarfing genes can be considered rather similar (Gale & Youssefian, 1985) and dependent on genetic background. Ellis *et al.* (2002) found that the *Rht-D1b* allele accounted for a larger proportion of phenotypic variance in plant height than *Rht-B1b*. The high preference for the *Rht-D1b* allele in the conditions of the Czech Republic (western Europe) can be explained by the germplasm used for crossing and by the better conditions for the desirable selection of 'tall-dwarfs' (Šíp *et al.* 1988).

Undoubtedly, the most important indication of the value of a cultivar is its area grown. It is generally

known that not only yielding ability but also grain quality and resistance to the important biotic and abiotic stresses are decisive for cultivar registration and spread. Table 3 shows that 0.85 of the wheat acreage in the Czech Republic is occupied by cultivars with an identified composition of alleles, a relatively higher acreage is now sown to wheat cultivars possessing the alleles 174-bp (0.41) and *Ppd-D1b* (0.62), and that are responsive to GA (0.61). A low proportion of the sown area at present is occupied by the carriers of the 192-bp allele (0.07). It is interesting that three out of four cultivars carrying the rare 198-bp allele (which cannot be separated accurately from the central European 197-bp allele identified by Worland *et al.* 2001) are now on 0.19 of the area (the German cultivar Akteur on 0.11 and the Czech cultivars Meritto and Sulamit on the rest). This allele can be considered as height promoting, because the average plant height of these cultivars was 0.99 m, while it was 0.92, 0.91 and 0.91 m for the alleles 174, 165 and 192-bp, respectively (Šíp *et al.* 2010). On the other hand, in Slovakia, (*Rht8*)192-bp and *Ppd-D1a* cultivars are still widely grown (on more than half the area), but at present a relatively small area is sown to GA-insensitive genotypes (0.10). GA responsiveness and the presence of (*Rht8*)192-bp and *Ppd-D1a* are characteristics of the most widespread modern Slovak cultivars Armelis, Axis and Bonita, and probably also Alacris, coming from the cross between Hana and Zerda.

A decline in the utilization of *Rht-B1b* and *Rht-B1d* in winter wheat breeding, as well as the absence of *Rht-D1b* in Slovak cultivars and a relatively low frequency of this GA-insensitive gene in modern cultivars of Czech origin, can, to a certain extent, be ascribed to problems with grain quality and winter hardiness (Šíp *et al.* 2010) that probably still exist. It has been demonstrated with spring wheat *Rht* isolines (Chapman *et al.* 2007) that over a large sample of environments tall isolines may yield the same or better than short lines, because in recent genetic backgrounds the tall isolines are shorter than their predecessors as a consequence of direct or indirect selection for several minor genes for reduced height (Trethowan *et al.* 2001). In winter wheat, the requirements for obtaining good bread-making quality and a high winter-hardiness level in combination with high and stable grain yield can probably be achieved better with GA responsiveness than GA insensitivity. This is because the GA-insensitive dwarfing genes *Rht-B1b* and *Rht-D1b* are environmentally sensitive (Worland *et al.* 2001), and there may be also problems with plant establishment, due to a shortening of coleoptile length, and relatively lower initial growth performance (Rebetzke *et al.* 2005; Richards *et al.* 2007; Tambussi *et al.* 2007). A preference for selecting GA-sensitive genotypes can be explained by desirable positive effects on cell size (greater leaf area development (LAD)) and high early vigour, opening new avenues for increasing

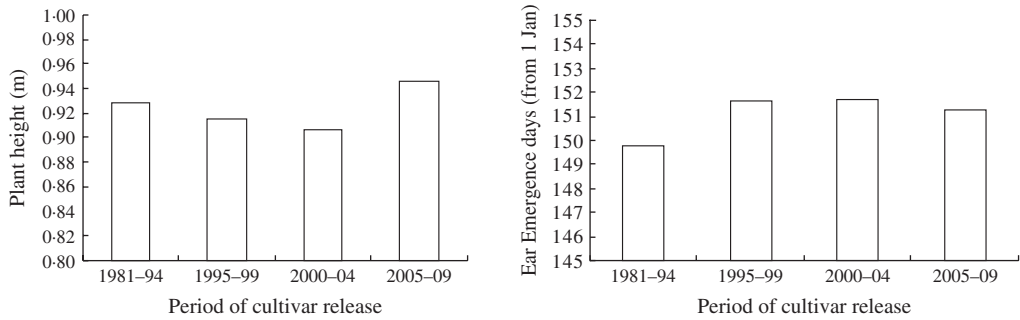


Fig. 5. Changes in mean plant height and ear emergence in four periods of cultivar release (1: 1981-94; 2: 1995-99; 3: 2000-04; 4: 2005-09).

water use efficiency (Botwright *et al.* 2005; Tambussi *et al.* 2007). According to recent findings (Holzapfel *et al.* 2008), a decline in the utilization of *Rht-D1b* can also be attributable to greater Fusarium head blight susceptibility.

Figure 5 shows the changes in average plant height and ear emergence in the four time periods. It is evident that there was a substantial decline in plant height around 2000 then, more recently, a rapid increase. In comparison with older cultivars, the currently used cultivars are later in heading, although some earlier types are still present. Preferential selection for taller and later flowering cultivars could be justified by better establishment under variable sowing conditions, a deeper rooting system, and a longer period for grain yield formation (higher LAD), resulting in better yielding ability and adaptability to different conditions. The comparison of *Ppd-D1a* and *Ppd-D1b* NILs by Foulkes *et al.* (2004) showed that the later flowering *Ppd-D1b* genotypes appeared to be able to extract more soil water during the season and largely maintain water uptake during grain filling under late drought compared to *Ppd-D1a* genotypes. It is assumed that the extended period to flowering may favour the development of a larger, more extensive, root system. This property is improved by *Ppd-D1b*, and could be more advantageous than early flowering and drought escape, a strategy adopted in southern European countries. However, the preference for *Ppd-D1a* is characteristic of a very successful breeding programme at Uhřetice, which is generally oriented towards the development of relatively taller cultivars than the other programmes in the Czech and Slovak Republics. The widespread use of the cultivars Alana, Alka, Mladka, Meritto and Bohemia, carrying *Ppd-D1a* mostly in combination with the *Xgwm261* allele 174-bp, shows that earlier cultivars can be advantageous for these conditions, provided plants are taller and their root system is highly developed. A widely grown tall (1.10 m) and early bread wheat cultivar Bohemia (Bobková & Hromádka 2008) can indicate a possible future preference for *Ppd-D1a* and

alleles contributing to greater plant height, provided the culm is stiff and lodging resistance is also achievable. Satisfactory stem strength characteristics are, therefore, highly desirable. Addisu *et al.* (2010) found that the presence of *Ppd-D1a* was beneficial for early resource capture (early season growth), which is highly desirable under organic growing systems. Reynolds *et al.* (2007) demonstrated a recent move from increased dry matter partitioning to grain towards increases of above-ground biomass by improving radiation use efficiency. Improving the balance between the source and sink is, according to those authors, currently the most promising approach for increasing yield, biomass and radiation use efficiency.

It can be implied from the recent wide exploitation of foreign, especially German, cultivars that broader adaptation and high yield potential are the most desirable properties. Šíp *et al.* (2010) demonstrated that broader adaptability to different growing conditions in a genotype can be created by combining *Xgwm261* alleles 174, 165 or 198-bp with *Ppd-D1b*. Indeed, obtaining broader adaptation in a genotype is undoubtedly becoming more and more important in all European regions owing to the relatively greater variation in weather conditions between years rather than between regions. However, there is danger that these genotypes will not be able to exploit certain growing conditions most effectively. Therefore, as stated by Reynolds & Borlaug (2006), a more complete knowledge of target environments is strategically useful in terms of determining which new traits and genes may be useful and the appropriate allocation of resources. Finding an optimum plant type (ideotype) is undoubtedly the most important starting point and orientation to certain allelic combinations can be considered as more strategically promising than seeking improvement (historical patterns) in single yield components (Sener *et al.* 2009).

In conclusion, the environments under study, in which different European wheat cultivars were exploited, can be considered as representative of the dominant wheat growing areas in Europe, and it can

be expected that the results obtained will have broader applicability, especially for appropriate use of certain *Rht* and *Ppd* allelic combinations and modelling the optimum plant type for certain growing conditions. Monitoring of evolutionary changes during a 30-year period helped to identify the progressive decline and increase in frequency of certain alleles and to identify allelic combinations that may be indicative of improved adaptability to different growing conditions. According to these results, a steady decline in utilization of the GA-insensitive semi-dwarfing genes and a preference for the *Xgwm261* alleles different from 192-bp can be expected in the future. The presence of the allele 192-bp (*Rht8*) can be recommended only in warmer (maize) growing regions. Improved properties (broader adaptation, suitability for organic and low-input agriculture) can be seen in the combination of the *Ppd-D1a* allele with height neutral or height-

promoting *Xgwm261* alleles. However, genetic studies of lodging resistance in these plant types are highly desirable. Broad adaptation under variable growing conditions of Central Europe is more likely achievable with the most frequent combination of *Ppd-D1b* and 174-bp *Xgwm261* allele. Useful sources of these alleles can be found in pedigree schemes that are presented in this paper. In general, this study can help in optimizing the breeding strategy and detect regions where certain cultivar types can perform well, because it is undoubtedly highly desirable to find the best use for the different arrays of germplasm produced in international breeding programmes.

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