Theor Appl Genet (1994) 89:179–184

M. Yamamori · T. Nakamura · T. R. Endo
T. Nagamine

Waxy protein deficiency and chromosomal location of coding genes in common wheat

Received: 26 October 1993 / Accepted: 21 December 1993

Abstract Deficiency of the wheat waxy (Wx) proteins (Wx-A1, Wx-B1 and Wx-D1) was studied in 1,960 cultivars derived from several countries. Gel electrophoretic analyses revealed that the null allele for the Wx-A1 protein occurred frequently in Korean, Japanese and Turkish wheats but was relatively rare in cultivars from other countries and regions. About 48% of the wheats deficient for the Wx-B1 protein were from Australia and India. One Chinese cultivar lacked the Wx-D1 protein. While 9 Japanese cultivars were deficient in both the Wx-A1 and Wx-B1 proteins, no cultivars lacked both the Wx-A1 and Wx-D1 proteins, both the Wx-B1 and Wx-D1 proteins or all three Wx proteins. Two-dimensional gel electrophoresis revealed polymorphisms of the three Wx proteins that varied according to isoelectric points or molecular weight. The *Wx-A1* gene coding the Wx-A1 protein and the *Wx-B1* gene coding the Wx-B1 protein were localized in the distal regions of chromosome arms 7AS and 4AL, respectively, by deletion mapping using the deletion lines developed in the common wheat cultivar 'Chinese Spring'.

Key words Waxy (Wx) protein · *Triticum aestivum* · Null allele · Geographical distribution · Chromosomal location

Introduction

Starch-granule bound or waxy (Wx) protein is involved in the amylose synthesis of endosperm starch (Tsai 1974; Echt and Schwartz 1981). Recent studies (Fujita et al. 1991; Yamamori et al. 1992; Nakamura et al. 1993c) have shown that the major starch-granule bound protein present in the endosperm of common wheat (*Triticum aestivum* L.) corresponds to the Wx protein of maize (Echt and Schwartz 1981) and rice (Sano 1984), which is the product of the *waxy* gene (Shure et al. 1983; Hirano and Sano 1991).

The major portion of wheat flour consists of storage starch, which is made up of amylopectin (ca. 74%) and amylose (ca. 25%), with minute amounts of lipid, etc. (Schofield and Greenwell 1987). Since the Wx protein level of the starch granules in wheat correlates to its amylose content (Yamamori et al. 1992), which in turn affects the glutinosity of Japanese 'udon' noodles (Oda et al. 1980), this protein is likely to play as an important role in flour quality as it does in rice (Sano et al. 1985) and maize (Imam 1989). Glutinosity is one of the factors responsible for the palatability of Japanese noodles in which some degree of 'stickiness' in desirable.

Because of allohexaploidy, common wheat has three Wx proteins, i.e. the Wx-A1, Wx-B1 and Wx-D1 proteins, which are coded by homoeologous genes located on the group 7 chromosomes (Chao et al. 1989; Nakamura et al. 1993a): the *Wx-A1* gene coding for the Wx-A1 protein is on chromosome arm 7AS, the *Wx-B1* gene on 4AL (a part of 7BS is translocated) and the *Wx-D1* gene on 7DS. In our studies, we have called the three alleles producing the Wx proteins in cv 'Chinese Spring' *Wx-A1a*, *Wx-B1a* and *Wx-D1a*.

A modified SDS polyacrylamide gel electrophoresis (SDS-PAGE) containing a low concentration of BIS acrylamide separated the wheat Wx proteins into two bands, a high-molecular-weight (HMW) band consisting of the Wx-A1 protein and a low-molecular-weight (LMW) band comprising the Wx-B1 and Wx-D1 proteins (Nakamura et al. 1992). Subsequently, the two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) succeeded in identifying the Wx proteins as three subunit groups (Nakamura et al. 1993a). These two electrophoretic techniques revealed that some of the

Communicated by G. Wenzel

M. Yamamori (✉) · T. Nagamine
Okinawa Sub-tropical Station, Japan International Research Center for Agricultural Sciences, Ishigaki, Okinawa 907, Japan

T. Nakamura
Tohoku National Agricultural Experiment Station, Morioka, Iwate 020-01, Japan

T. R. Endo
Nara University, 1500 Misasagi-cho, Nara 631, Japan

improved Japanese cultivars lack one or both of the Wx-A1 and Wx-B1 proteins (Nakamura et al. 1992, 1993a, b).

This paper will describe the geographical distribution of the Wx protein deficiency in wheat cultivars, the discovery of a null allele for the Wx-D1 protein and polymorphism of the three Wx proteins. In addition, our investigation located the *Wx-A1* and *Wx-B1* loci on cytological maps using the deletion stocks of cv 'Chinese Spring', which have various degrees of deficiency in particular chromosome arms (Endo and Gill 1993).

Materials and methods

Wheat materials

We used a total of 1,960 wheat cultivars (Table 1), most of which were provided by the National Institute of Agrobiological Resources (NIAR) of the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF), and the rest by MAFF wheat breeding laboratories. Cultivars originated from 11 countries and geographical regions, including North America (USA and Canada) and Western Europe (UK, FRG, France and Italy).

In order to locate the *Wx-A1* and *Wx-B1* loci on cytological maps, deletion stocks of cv 'Chinese Spring' developed by Endo and Gill (1993) were used. For the *Wx-A1* locus, 6 stocks for chromosome arm 7AS (lines 7AS-1, -2, -3, -5, -9 and -12) were analyzed and for the *Wx-B1* locus, 9 stocks for 4AL (lines 4AL-1, -2, -4, -5, -6, -7, -10, -11 and -14) were examined. No homozygous deletion lines for chromosome arm 7DS, on which the *Wx-D1* gene is located, were available.

Gel electrophoretic techniques

To examine the wheat Wx proteins, we used two electrophoretic techniques. One was a modified SDS-PAGE using an acrylamide/BIS concentration of 30:0.135, which was applied to all the materials (Table 1). This method can distinguish the Wx-A1 protein (HMW band) from the Wx-B1 and Wx-D1 proteins forming the LMW band. The other technique, which was applied to 1,551 cultivars (Table 1), was a 2D-PAGE in which isoelectric focusing (IEF) was used for the first dimensional electrophoresis and the modified SDS-PAGE for the second dimensional. The positions of three Wx proteins on the gel followed the diagram of Nakamura et al. (1993a). Extraction of the Wx protein and electrophoresis were conducted according to the procedures described by Nakamura et al. (1992, 1993a) except that a 12.5% (w/v) concentration of polyacrylamide gel was used for the modified SDS-PAGE. Starches for extracting the Wx protein were prepared from ten wheat grains. To detect the protein, Coomassie Brilliant Blue (CBB) and silver stain were applied to the modified SDS-PAGE and CBB to the 2D-PAGE.

Table 1 Distribution of wheat cultivars lacking the Wx-A1, Wx-B1 and Wx-D1 proteins as revealed by modified SDS-PAGE and 2D-PAGE

Origin	Number of cultivars			
	Examined[a]	Lacking the Wx protein		
		Wx-A1	Wx-B1	Wx-D1
Japan	462 (333)	75 (16.2%)	16	0
South & North Korea	93	10 (10.8)	1	0
China	308 (171)	3 (1.0)	12	1
India	50	3 (6.0)	25	0
Pakistan	85	0 (0.0)	13	0
Afghanistan	59	0 (0.0)	13	0
Turkey	156	81 (51.9)	0	0
Australia	127	1 (0.8)	51	0
North America	315 (172)	3 (1.0)	19	0
Western Europe	172	1 (0.6)	4	0
Russia	133	0 (0.0)	5	0
Total	1,960 (1,551)	177 (9.0%)	159	1

[a] All cultivars (1,960) were subjected to the modified SDS-PAGE to determine the degree of deficiency of the Wx-A1 protein. 2D-PAGE was applied to 1,551 cultivars (333 Japanese, 171 Chinese, 172 North American, and all the rest of the cultivars) to determine the degree to which the Wx-B1 and Wx-D1 proteins were deficient

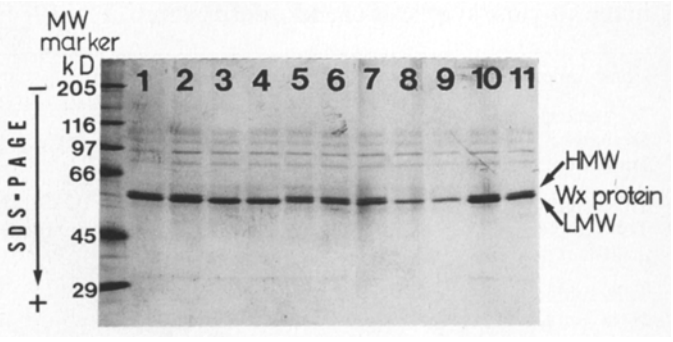
Results

Geographical distribution of wheat cultivars deficient for the Wx proteins

While most cultivars generated both the HMW and LMW bands by the modified SDS-PAGE (Fig. 1), 177 cultivars (9.0%) did not produce the HMW band (= Wx-A1 protein) in either CBB- or silver-stained gels, showing that they had a null allele for the Wx-A1 protein (termed *Wx-A1b*) (Table 1). This null allele, *Wx-A1b*, occurred frequently in Turkish (51.9%), Japanese (16.2%) and Korean (10.8%) cultivars, but only rarely in those from other countries and regions (0.0–6.0%). The allele *Wx-A1b* was not found in any of the Pakistani, Afghan or Russian cultivars examined.

In cultivars having both the HMW and LMW bands, the latter band was usually thicker than the former. Some cultivars, however, showed a thinner LMW band, and its thickness became almost the same as that of the HMW band (Lanes 5 and 6 in Fig. 1). Since the LMW band comprises both the Wx-B1 and Wx-D1 proteins, these cultivars might have lacked one of the two proteins. The succeeding 2D-PAGE revealed that almost all of them were lacking the Wx-B1 protein but none lacked

Fig. 1 Variation of wheat Wx proteins examined by the modified SDS-PAGE. *Lane numbers* correspond to the cultivar numbers in Table 2. Proteins were stained with Coomassie Brilliant Blue. Lanes 3, 4, 8 and 9 did not produce the HMW band. While the LMW band was thicker than the HMW in lanes 1, 2, 7, 10 and 11, the thickness of the two bands was similar in lanes 5 and 6



the Wx-D1 protein. In a few cultivars, the level of the Wx-B1 protein seemed to be reduced. To examine whether any of the cultivars showing a thick LMW and a thin HMW band possessed the null allele for either the Wx-B1 or Wx-D1 protein, the 2D-PAGE was further performed on about 1,200 cultivars. However, no cultivars lacked the Wx-B1 protein in this survey. Finally, we found that 159 cultivars carried the null allele for the Wx-B1 protein (*Wx-B1b*) (Table 1), of which 51 cultivars were from Australia and 25 were from India. Thus, cultivars from these two countries accounted for about one-half (47.8%) of the cultivars carrying *Wx-B1b*. The *Wx-B1b* null allele was distributed over all countries and regions except Turkey.

Concerning the deficiency of the Wx-D1 protein, we found that Chinese cv 'Bai Huo', which showed a LMW band that was slightly thicker than its HMW band, did not produce the Wx-D1 protein (Table 1, Fig. 2). This null allele was designated *Wx-D1b* (Table 2).

Next we examined wheats lacking the two Wx proteins. The nonexistence of cultivars lacking the LMW band provided evidence that none lacked both the Wx-B1 and Wx-D1 proteins. To determine whether cultivars deficient for the Wx-A1 protein also lacked the Wx-B1 or Wx-D1 protein, 177 cultivars carrying the *Wx-A1b* allele in Table 1 were further subjected to 2D-PAGE. Two Japanese cultivars included in this study, 'Kanto 79' and 'Kanto 107', had been shown earlier to be deficient in both the Wx-A1 and Wx-B1 proteins (Nakamura et al. 1993b). In addition, 7 cultivars ('Hakufu Yuubou', 'Kanto 115', 'Saikai 170', '171', '172', '173' and 'Norin 67') also lacked the two Wx proteins and produced a thin LMW band. Four 'Saikai' lines and 'Kanto 115' have been bred using 'Kanto 107' as a crossing parent. The remaining 168 cultivars had both the Wx-B1 and Wx-D1 proteins, indicating that they lacked only the Wx-A1 protein.

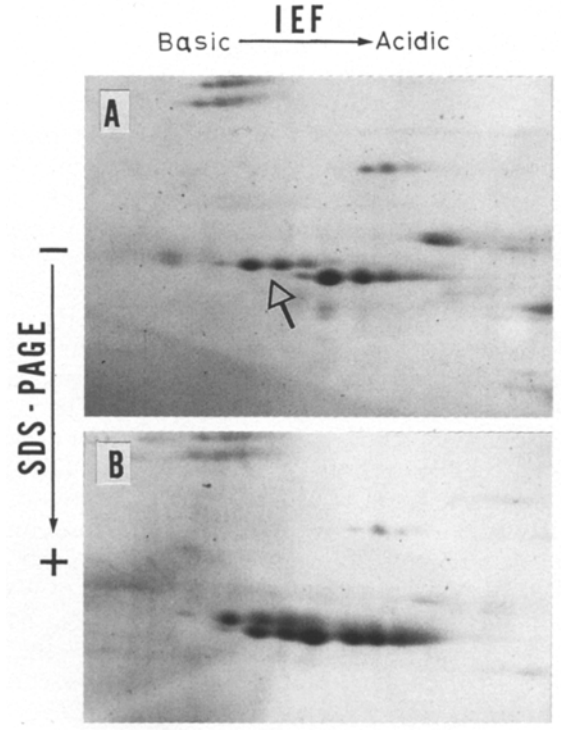
Consequently, 9 Japanese cultivars lacked both the Wx-A1 and Wx-B1 proteins, but cultivars which lacked both the Wx-B1 and Wx-D1, both the Wx-A1 and Wx-D1 or all three Wx proteins were not found in the germplasm examined.

Table 2 Alleles of *Wx-A1*, *Wx-B1* and *Wx-D1* loci in wheat cultivars

Number	Cultivar (origin)	Allele			
		Wx-A1	*Wx-B1*	*Wx-D1*	Type[a]
1	Chinese Spring (China)	*a*	*a*	*a*	1
2	Norin 61 (Japan)	*a*	*a*	*a*	1
3	Shirodaruma (Japan)	*b*	*a*	*a*	2
4	Sturdy (USA)	*b*	*a*	*a*	2
5	Gabo (Australia)	*a*	*b*	*a*	3
6	Satanta (USA)	*a*	*b*	*a*	3
7	Bai Huo (China)	*a*	*a*	*b*	4
8	Kanto 107 (Japan)	*b*	*b*	*a*	7
9	Norin 67 (Japan)	*b*	*b*	*a*	7
10	Bezostaja (Russia)	*a*	*a*	*a*	1
11	Maris Huntsman (UK)	*a*	*a*	*a*	1
12	Pakistan Zairaishu QT 105 (Pakistan)	*c*	*a*	*a*	1
13	Pakistan Zairaishu WB 6 (Pakistan)	*c*	*a*	*a*	1
14	Junbuk 12 (Korea)	*a*	*c*	*a*	1
15	Cikotaba (Russia)	*a*	*c*	*a*	1
16	Scoutland (USA)	*a*	*a*	*c*	1

[a] See Fig. 5

Fig. 2A, B 2D-PAGE patterns of Chinese cv 'Bai Huo' lacking the Wx-D1 protein (**A**) and Japanese cv 'Norin 61' having three Wx proteins (**B**). The absence of the Wx-D1 protein in 'Bai Huo' is marked by an *arrow*



Polymorphism of wheat Wx proteins

During the survey of the materials, we found a few variants for each of the three Wx proteins. Their modified SDS-PAGE generated a thin HMW band and a thick LMW band (Fig. 3A).

Variants for the Wx-A1 protein were found in 7 Pakistani cultivars. The molecular weight of the altered Wx-A1 protein was slightly lower and its isoelectric point (pI) was a little more basic than that of the wild Wx-A1 protein (Fig. 3). This allele was designated *Wx-A1c* (Table 2). The altered Wx-B1 protein whose pI became a little more basic than that of the normal Wx-B1 (Fig. 3B) existed in 11 cultivars from 6 countries. This allele was designated *Wx-B1c* (Table 2). In the American cultivar 'Scoutland', the altered Wx-D1 protein possessed a pI that was a little more basic than that of the wild type (Fig. 3B). This allele was designated *Wx-D1c* (Table 2).

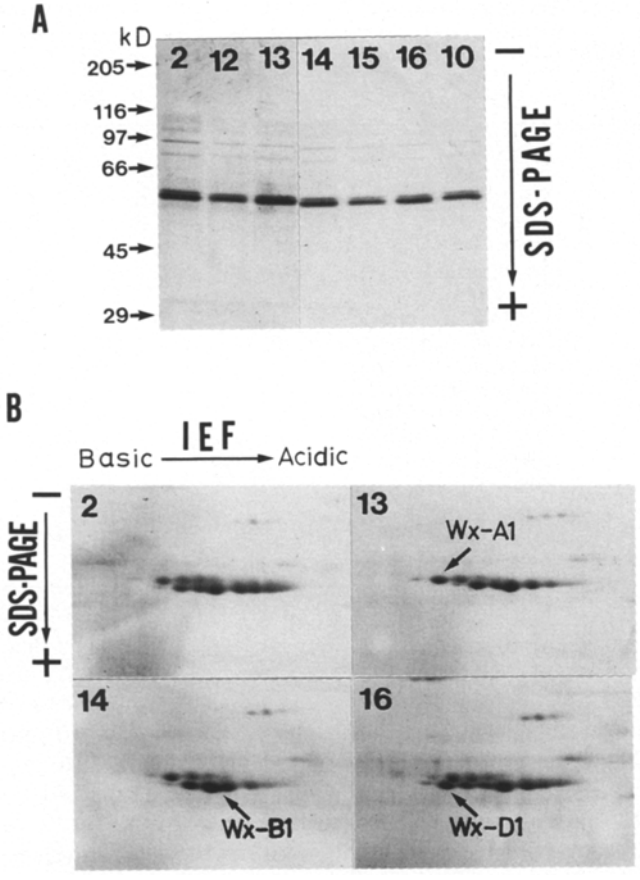


Fig. 3A,B SDS-PAGE (**A**) and 2D-PAGE (**B**) patterns of altered Wx proteins. *Numbers* on electrophoretic gels correspond to the cultivar numbers in Table 2. The altered Wx-A1 protein appeared in lanes 12 and 13, the altered Wx-B1 protein in lanes 14 and 15 and the altered Wx-D1 protein in lane 16. On the two-dimensional gels (**B**), the altered Wx proteins are indicated by *arrows* and the normal Wx proteins of cv 'Norin 61' (*B2*) are shown as a control

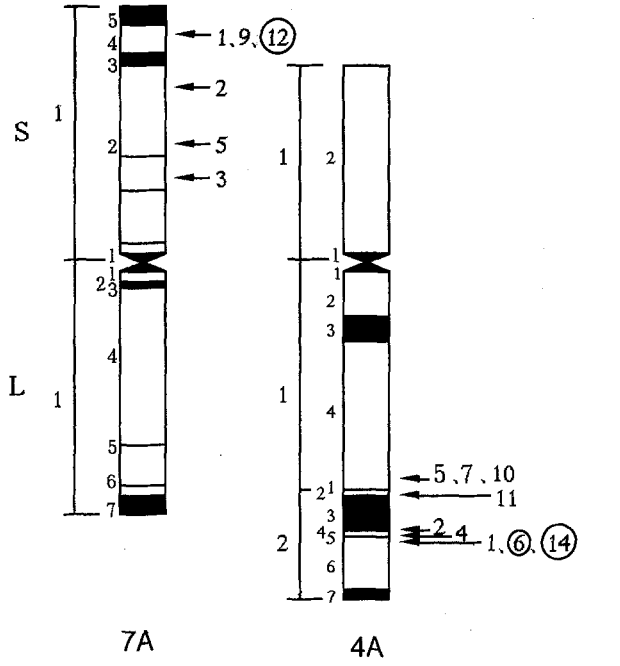


Fig. 4 Breakpoints (indicated by *arrows*) of deletion stocks on ideogram of banded chromosomes. The breakpoints of 7AS-3 and -5 relative to the two faint bands in the region S1.2 are conjectural. The *circled* stocks produced the Wx protein coded by the *Wx* gene on the chromosome arm, while the remaining stocks did not produce this protein. The ideogram is according to Gill et al. (1991)

Physical location of the *Wx-A1* and *Wx-B1* genes on chromosome maps

The breakpoints of the deletion stocks are indicated in Fig. 4 by arrows; on each chromosome arm, the segment distal from the breakpoint has been deleted.

Six deletion stocks for chromosome arm 7AS were examined to determine the *Wx-A1* locus on the cytological map. The modified SDS-PAGE showed that 5 lines (7AS-1, -2, -3, -5 and -9) lacked the Wx-A1 protein while 1 line (7AS-12) possessed it (Fig. 4). To determine the location of the *Wx-B1* gene, we carried out 2D-PAGE using 9 deletion stocks for chromosome arm 4AL. Though 2 lines (4AL-6 and -14) produced the Wx-B1 protein, the remaining 7 lines did not (Fig. 4). These results showed that both of the *Wx-A1* and *Wx-B1* loci were confined in very narrow regions because the breakpoints of 7AS-1, -9 and -12 and those of 4AL-1, -6 and -14 were not distinguishable by C-banding. Therefore, the *Wx-A1* and *Wx-B1* genes should be very close to the broken telomeric regions of the 7AS-12 and 4Al-6 or 4AL-14 chromosomes, respectively.

Discussion

Relationship between a deficiency of Wx proteins and amylose content

On the basis of the presence or absence of the three Wx proteins, wheat cultivars can be classified into eight types as shown in Fig. 5; types 1, 2, 3, 4 and 7 existed in the materials examined in our study. We had suggested earlier that the Wx protein level varied among 31 Japanese cultivars and was related to the amylose content of flour (Yamamori et al. 1992). According to the above criteria, we can group 133 Japanese cultivars whose apparent amylose content had been determined by Kuroda et al. (1989) into types 1, 2, 3 and 7 (Fig. 5). The amylose content tends to decrease in the order of type 1 (28.0 mg per 100 mg flour on average), type 2 (27.2 mg), type 3 (25.3 mg) and type 7 (22.0 mg). The loss of the Wx-B1 protein (type 3) seems to reduce amylose content rather than the loss of the Wx-A1 protein (type 2). The 2D-PAGE analysis revealed that the amount of Wx-B1 protein was larger than of the Wx-A1 protein as described by Nakamura et al. (1993a). Therefore, the lack of the Wx-B1 protein should afffect the amylose content more than the lack of the Wx-A1 protein. Consequently,

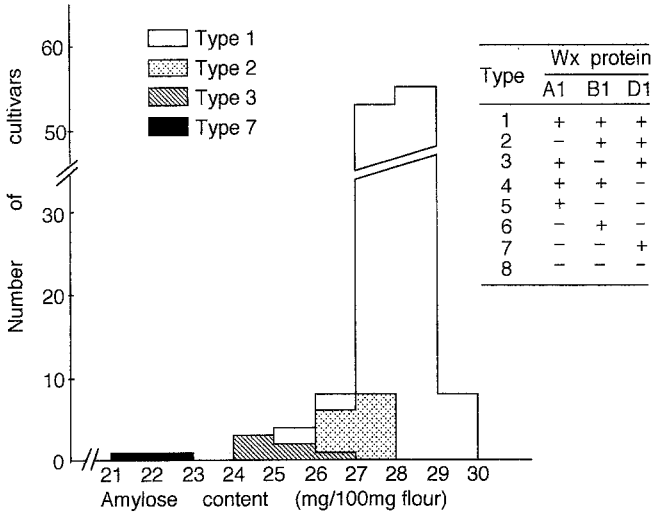


Fig. 5 Classification of apparent amylose contents of 133 Japanese cultivars on the basis of the presence or absence of the Wx proteins. Amylose contents (mg per 100 mg flour) were cited from Kuroda et al. (1989). + and − in eight types indicate the presence and absence of each Wx protein, respectively

the variations in the amount of Wx protein and amylose content previously reported have proved to be mainly explained by a partial deficiency of the Wx proteins.

The primary purpose of this survey was to find cultivars deficient in the Wx-D1 protein. This would allow us to cross type 7 Japanese cultivars with a type 4 Chinese cultivar to produce eight possible types of wheat (Fig. 5). This in turn would enable us to learn how much Wx protein and amylose are produced by these wheats, especially type 8.

The geographical distribution of null alleles

This study showed the geographical distribution of the three null alleles, *Wx-A1b*, *Wx-B1b* and *Wx-D1b* (Table 1).

Wx-A1b was frequent in Turkish cultivars. Similarly, Nakamura et al. (1990) reported that about three-quarters of the 150 Turkish cultivars they examined exhibited one HMW glutenin composition consisting of subunit bands 2 + 12 and 7 + 8. Their materials were obtained from the same institute (NIAR) as ours were. Thus, the Turkish stocks preserved are characterized by a high frequency of the *Wx-A1b* allele and comparatively homogeneous genotype of glutenin. Japanese and Korean cultivars contained the *Wx-A1b* allele at frequencies of 16% and 11%, respectively. Though this similarity can be attributed to the short distance between these two countries, it is difficult to explain the comparatively high frequencies.

About 40% of the Australian wheats carried the null allele *Wx-B1b* (Table 1). The Australian Standard White (ASW), a wheat brand composed of several Australian cultivars, shows a lower amylose content of flour and better glutinosity of noodles than standard Japanese wheats (e.g. 'Norin 61'). This survey revealed that some of the cultivars making up ASW (e.g. 'Halberd', 'Aroona', 'Gutha', 'Gamenya', 'Rosella') carried the *Wx-B1b*. Since the presence of the null allele *Wx-B1b* seemed to lower the amylose content to some extent as mentioned above, it would be also responsible for the low amylose level of ASW.

The occurrence of the null allele *Wx-D1b* was very rare; only one of 1,551 cultivars carried it (Table 1). On the other hand, the *Wx-A1b* occurred in 9.0% (177) of the 1,960 cultivars while the *Wx-B1b* was found in 10.3% (159) of 1,551 cultivars. The reason for the infrequency of *Wx-D1b* remains unknown.

Polymorphism of the Wx proteins

The *Wx-A1c*, *Wx-B1c* and *Wx-D1c* alleles produced Wx proteins whose pIs were a little more basic than those of the wild types. Terachi et al. (1988) suggested that possible amino acid changes deduced from the DNA sequences caused the difference in pI btween two types of the ribulose-1, 5-bisphosphate carboxylase (Rubisco) large subunit in common wheat and *Aegilops* species. This sort of mutation might have arisen in the alleles found in this study.

In a few materials, the Wx-B1 protein seemed to be reduced. Because of some overlapping of the subunit groups of the Wx-B1 and Wx-D1 proteins, we could not conclude whether or not this was caused by an allelic variation of *Wx-B1*. When the reduced Wx-B1 protein is extracted as type 6 or type 4 wheat on an electrophoretic gel, we can confirm the presence of an allele yielding a low level of Wx-B1 protein.

References

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor Appl Genet 78:495–504

Echt CS, Schwartz D (1981) Evidence for the inclusion of controlling elements within the structural gene at the waxy locus in maize. Genetics 99:275–284

Endo TR, Gill BS (1993) Production of deletion stocks in common wheat. In: Proc 8th Int Wheat Genet Symp, Beijing (in press)

Fujita N, Uematsu M, Taira T (1991) Relationship between SDS-PAGE pattern of *Wx* protein and various genomes in *Triticum*, *Secale* and tetraploid Triticale (in Japanese). Jpn J Breed 41 [Suppl 2]: 70–71

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). Genome 34:830–839

Hirano HY, Sano Y (1991) Molecular characterization of the *waxy* locus in rice (*Oryza sativa*). Plant Cell Physiol 32:989–997

Imam SH (1989) A tightly bound Mr 55000 polypeptide in cornstarch associated with the amylose portion of the granule. J Cereal Sci 9:231–236

Kuroda A, Oda S, Miyagawa S, Seko H (1989) A method of measuring amylose content and its variation in Japanese wheat cultivars

and Kanto breeding lines (in Japanese). Jpn J Breed 39 [Suppl 2]: 142–143

Nakamura H, Yamada T, Hirano H (1990) Geographical variation of glutenin subunit compositions of seed storage proteins in wheat cultivars (in Japanese). Jpn J Breed 40 [Suppl 2]: 220–221

Nakamura T, Yamamori M, Hidaka S, Hoshino T (1992) Expression of HMW Wx protein in Japanese common wheat (*Triticum aestivum* L.) cultivars. Jpn J Breed 42:681–685

Nakamura T, Yamamori M, Hirano H, Hidaka S (1993a) Identification of three Wx proteins in wheat (*Triticum aestivum* L.). Biochem Genet 31:75–86

Nakamura T, Yamamori M, Hirano H, Hidaka S (1993b) Decrease of waxy (Wx) protein in two common wheat cultivars with low amylose content. Plant Breed 111:99–105

Nakamura T, Yamamori M, Hirano H, Hidaka S (1993c) Characterization of three waxy (Wx) proteins in wheat (*Triticum aestivum* L.). In: Proc 8th Int Wheat Genet Symp, Beijing (in press)

Oda M, Yasuda Y, Okazaki S, Yamauchi Y, Yokoyama Y (1980) A method of flour quality assessment for Japanese noodles. Cereal Chem 57:253–254

Sano Y (1984) Differential regulation of waxy gene expression in rice endosperm. Theor Appl Genet 68:467–473

Sano Y, Katsumata M, Amano E (1985) Correlations between the amounts of amylose and Wx protein in rice endosperm. SABRAO J 17:121–127

Schofield JD, Greenwell P (1987) Wheat starch granule proteins and their technological significance. In: Morton ID (ed) Cereals in a European context. Ellis Horwood, Chichester, pp 407–420

Shure M, Wessler S, Fedoroff N (1983) Molecular identification and isolation of the *waxy* locus in maize. Cell 35:225–233

Terachi T, Ogihara Y, Tsunewaki K (1988) The *rbcL*genes in wheat and several *Aegilops* species with divergent chloroplast genomes. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp. Bath Press, Bath, UK, pp 789–795

Tsai CY (1974) The function of the waxy locus in starch synthesis in maize endosperm. Biochem Genet 11:83–96

Yamamori M, Nakamura T, Kuroda A (1992) Variations in the content of starch-granule bound protein among several Japanese cultivars of common wheat (*Triticum aestivum* L.). Euphytica 64:215–219