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Variation of starch granule proteins and chromosome mapping of their coding genes in common wheat

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Abstract Starch granule proteins (SGPs) of common wheat (*Triticum aestivum* L.) were analyzed by two electrophoretic techniques: sodium dodecyl sulphate polyacrylamide-gel electrophoresis (SDS-PAGE) and two-dimensional electrophoresis (2D-PAGE). These analyses identified three kinds of SGPs which were tentatively designated SGP-1, SGP-2 and SGP-3. SDS-PAGE resolved the products of three homoeologous genes for SGP-1 into three protein fractions, SGP-A1, -B1 and -D1. While SDS-PAGE resolved SGP-3 into one fraction, 2D-PAGE separated it into three protein fractions encoded by homoeologous genes *Sgp-A3*, *-B3* and *-D3*. SGP-2 was detected as one protein by SDS-PAGE and was present as one protein on 2D-PAGE. Aneuploid (nullisomic-tetrasomic and ditelosomic) analyses in the cultivar Chinese Spring showed that the genes for two SGPs (SGP-1 and -3) were located on the short arms of group-7 chromosomes. The results obtained from deletion lines for chromosome arms 7AS, 7BS and 7DS suggested that the gene order along the arms is 'centromere–*Sgp-1*–*Sgp-3*–*Wx*'. An electrophoretic survey of wheat germ plasm identified a few cultivars lacking one of the proteins SGP-A1, -B1, -D1, SGP-A3 and -B3. The null alleles *Sgp-A1b*, *Sgp-B1b* and *Sgp-D1b* will be useful for the production of a variant wheat lacking SGP-1.

Key words Starch granule proteins · *Triticum aestivum* L. · Gel electrophoresis · Chromosome mapping · Variation

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Introduction

Flour of common wheat (*Triticum aestivum* L.) is composed of about 11% protein, 67% starch, 13% water and 9% miscellaneous materials. While the majority of flour protein is composed of glutenin, gliadin, albumin and globulin, some minor proteins are retained by water-washed starch granules of flour (Lowy et al. 1981; Greenwell and Schofield 1986; Skerritt et al. 1990). Using sodium dodecyl sulphate polyacrylamide-gel electrophoresis (SDS-PAGE), Schofield and Greenwell (1987) detected ten starch granule proteins (SGPs) whose molecular masses ranged from 5 to 149 kDa. They classified these proteins into surface SGPs and integral SGPs according to their extractability from starch granules. Five integral SGPs of 59–149 kDa were solubilized from starch swollen in a heated (>50° or 100°C) protein extraction buffer containing SDS, showing that these proteins were tightly bound to starch granules (Schofield and Greenwell 1987). Among the integral SGPs of wheat, the major SGP (59 or 61 kDa) is the product of the *waxy* gene and is called the waxy (Wx) protein (Ainsworth et al. 1993; Nakamura et al. 1993 b). The Wx protein is known to be a granule-bound starch synthase responsible for amylose production (Preiss 1991). However, the other integral (tightly-bound) SGPs which show higher molecular masses than the Wx protein have not been examined in detail.

Recent studies have indicated that the starch synthase and starch branching enzyme are bound to starch granules. Denyer et al. (1993) showed that three isoforms of the starch synthase and starch-branching enzyme (77–114 kDa) from pea embryos were soluble and starch granule-bound. In maize, a 76-kDa protein, immunologically similar to soluble starch synthase (SSS) I, appeared to be tightly associated with the starch granules of the endosperm (Mu et al. 1994). Further, Denyer et al. (1995) indicated an association of the starch synthase or branching-enzyme activity with the granule-bound proteins in developing wheat endosperm. These findings suggest that some soluble isoforms of the starch synthase and branching enzyme become

trapped within starch as the starch granule grows (Denyer et al. 1993).

In our study of the wheat Wx protein, a highly sensitive silver-staining method detected a few starch granule-bound proteins (integral SGPs) with higher molecular masses than the Wx protein (61 kDa) on SDS-PAGE (Yamamori et al. 1992). The presence of such proteins was also reported in studies of the Wx proteins of maize, rice, and other grains (Echt and Schwartz 1981; Sano 1984; Goldner and Boyer 1989). The present report describes the electrophoretic analyses of the integral SGPs of common wheat, the chromosomal locations of their genes, and the diversity of the proteins.

Materials and methods

Plant materials

To determine gene locations on chromosome arms, we used the aneuploid [nullisomic-tetrasomic (NT) and ditelosomic (Dt)] lines of a common wheat cultivar, Chinese Spring, produced by Sears (1966) and by Sears and Sears (1978), respectively; NT lines lacking chromosomes 2A and 4B were not available. To determine gene order on the chromosomes, we used the deletion lines of Chinese Spring developed by Endo and Gill (1993). We analyzed 11 homozygous deletion stocks for chromosome arm 7AS, three homozygous stocks for 7BS and four homozygous stocks for 7DS. C-banding analysis identified various degrees of terminal deletion in these chromosome arms (Endo and Gill 1993; Hohmann et al. 1994). A total of 1960 common wheat cultivars from 11 countries and geographical regions were obtained from the wheat breeding laboratories of the Japanese Ministry of Agriculture, Forestry and Fisheries. As a control, we examined a rice cultivar, Nipponbare, and a maize cultivar, Heigenminori.

Electrophoresis of integral starch granule proteins (SGPs)

Endosperm starches of mature wheat grains were prepared using SDS extraction buffer which consisted of 55 mM Tris/HCl, pH 6.8, 2.3% SDS, 5% 2-mercaptoethanol and 10% glycerol (Echt and Schwartz 1981). Starches were washed three times with the SDS buffer, twice with distilled water, and then twice with acetone. The integral (tightly bound) SGPs for SDS-PAGE were solubilized from 2.5 mg of starch in 50 μl of SDS extraction buffer at 90°C for 5 min. After centrifugation, 20 μl of the supernatant was subjected to a modified SDS-PAGE in which a low BIS-acrylamide concentration (acrylamide/BIS concentration of 30:0.135) and an acrylamide concentration of 12.5 or 10% were used for the resolution gel (Kagawa et al. 1988).

For two-dimensional polyacrylamide-gel electrophoresis (2D-PAGE), 8.0 mg of starch was swollen at room temperature in 300 μl of lysis buffer [8 M urea, 2% Nonidet-P40, 2% ampholine pH 3.5–10 (Pharmacia LKB) and 5% 2-mercaptoethanol]. After centrifugation, the supernatant containing the solubilized SGPs was subjected to 2D-PAGE using isoelectric focusing (IEF) for the first dimension and modified SDS-PAGE for the second (Nakamura et al. 1993 a). IEF gels contained 2.5% (v/v) ampholines (pH 3.5–10/5–8, 1:1). Focusing was begun from the acidic end (0.01 M H_3PO_4) and continued at 200 V for 15 h, then 400 V for 50 min at room temperature.

The proteins separated on electrophoretic gels were detected by silver staining (Silver stain kit. Wako Pure Chemical Industries, Ltd.).

Results

Separation of starch granule proteins (SGPs) by SDS-PAGE and 2D-PAGE

In Chinese Spring, modified SDS-PAGE generated five bands of SGPs showing slower migrations and lower intensities than the Wx protein (molecular mass 61 kDa) (Fig. 1 A). Both the rice and maize cultivars produced three thin SGP bands above the thick band of the Wx protein.

Neither nullisomic 7A-tetrasomic 7B (N7A-T7B) nor N7A-T7D produced the 115-kDa protein (Fig. 1 B), but the other NT lines did. This indicates that the gene encoding the 115-kDa protein is located on chromosome 7A. Similarly, using the NT lines, the gene for the 100-kDa protein was shown to be located on chromosome 7B and the gene for the 108-kDa protein on 7D. Telosomic analysis using five ditelosomic (Dt) lines (7AS, 7AL, 7BS, 7BL, and 7DS) revealed that the genes for three SGPs were on the short arms 7AS, 7BS and 7DS, because Dt7AL lacked the 115-kDa SGP, Dt7BL lacked the 100-kDa protein, whereas all the other Dt lines produced all three proteins.

As all the NT lines examined showed bands corresponding to the 80-kDa and 92-kDa SGPs, multiple genes on different chromosomes appear to control the production of each SGP or else the responsible genes may be on chromosome 2A or 4B for which NT lines were not available for this study.

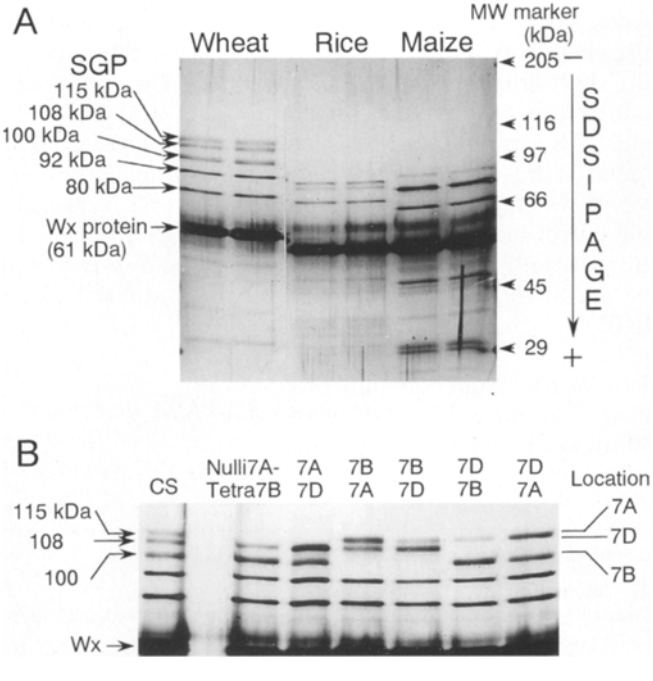

Fig. 1 A, B SDS-PAGE patterns of integral starch granule proteins (SGPs) in wheat cultivar Chinese Spring (*CS*), rice cv Nipponbare, and maize cv Heigenminori (**A**). Nullisomic-tetrasomic analysis of SGPs by SDS-PAGE (**B**). Nullisomy for each of the group-7 chromosomes caused deficiency in one of the 115-, 108- or 100-kDa SGPs

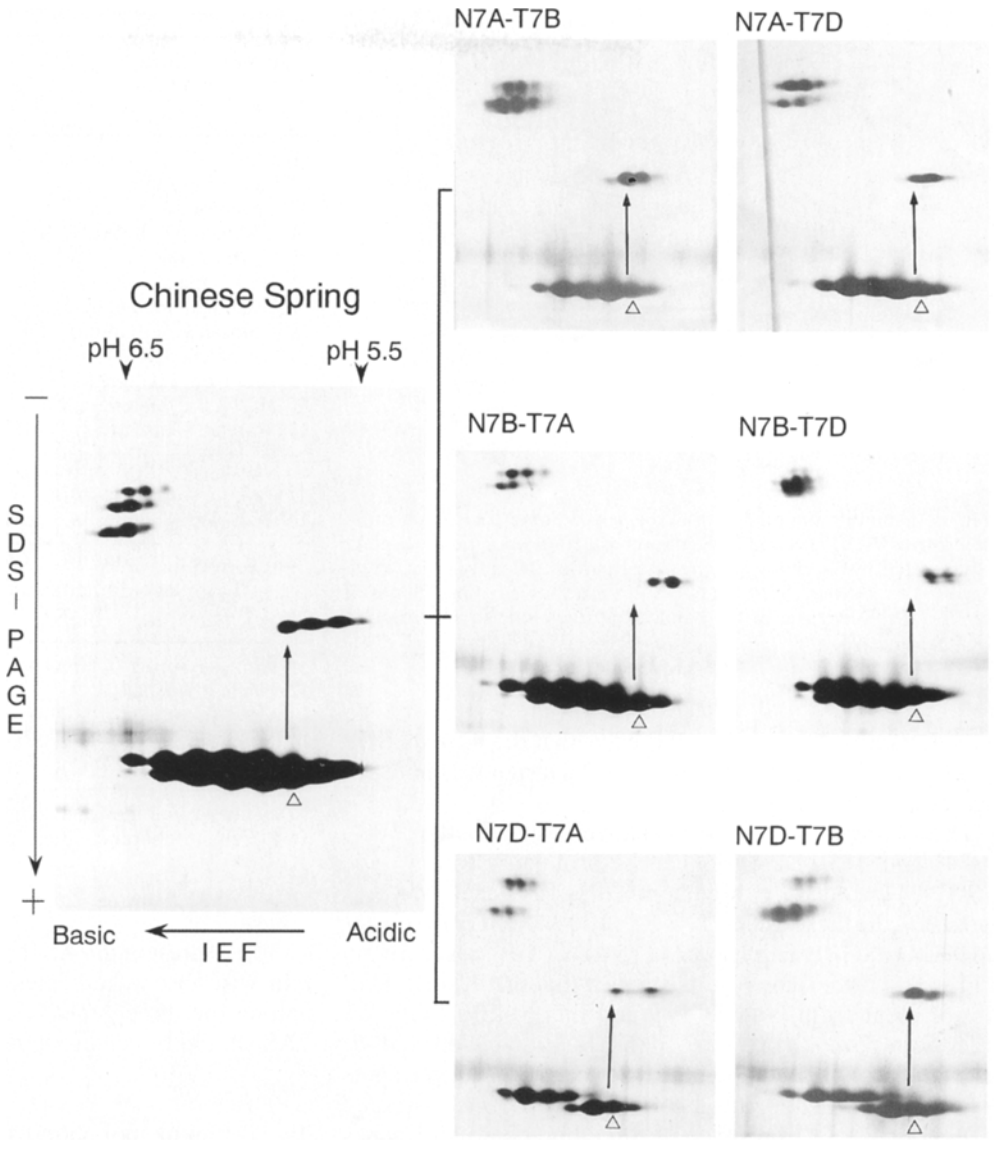

Fig. 2 2D-PAGE pattern for SGPs and nulli-tetrasomic analysis of the 80-kDa SGP in Chinese Spring. *Arrows* indicate the positions of the most basic 80-kDa SGP spot (SGP-B3) and *triangles* (Δ) indicate the same positions of the Wx-B1 protein (see also Fig. 3). In each NT line, the lack of a SGP-3 spot was caused by nullisomy for one of the group-7 chromosomes, while a larger SGP-3 spot resulted from tetrasomy for another group-7 chromosome

Of the above five SGPs bands, 2D-PAGE detected four proteins (80, 100, 108, 115 kDa) (see Figs. 2 and 3). Three SGPs (100, 108, and 115 kDa) showed almost the same isoelectric points (pIs) as the subunit group of the Wx-A1 protein. The 80-kDa SGP was detected as three small spots with pI values which were similar to that of the Wx-B1 protein. Nulli-tetrasomic (Fig. 2) and telosomic analyses revealed that the gene encoding the most basic spot of the 80-kDa SGP was located on 7BS, the gene for the middle spot on 7DS, and that for the most acidic was on 7AS. On the other hand, the 92-kDa SGP was not seen on 2D-PAGE. However, when the ampholine (pH 5–8) used for IEF gels was replaced by a more-acidic ampholine (pH 3.5–5), the 92-kDa SGP was detected as one spot at the most acidic

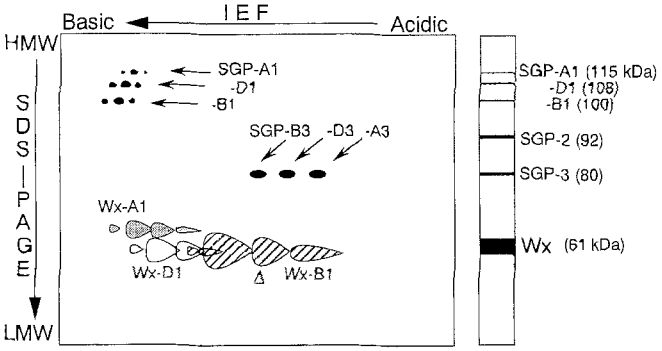

Fig. 3 SDS-PAGE and 2D-PAGE diagrams of the wheat integral SGPs. The position of the *triangle* (Δ) in Wx-B1 corresponds to those in Figs. 2 and 6

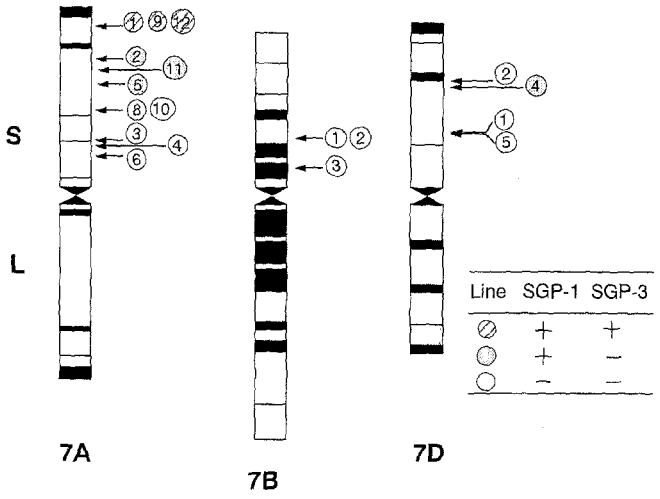

Fig. 4 Deletion mapping of the *Sgp-1* and *Sgp-3* loci on chromosome arms 7AS, 7BS and 7DS. In each deletion line, the breakpoint is indicated by an *arrow* and the distal part of the arm is lost. Presence (+) or absence (−) of the two SGPs are shown by three kinds of *circles*. The ideogram of the banded chromosomes is according to Gill et al. (1991)

Table 1 *Sgp-1* alleles in wheat cultivars

Cultivar (origin)	Allele		
	Sgp-A1	*Sgp-B1*	*Sgp-D1*
1 Chinese Spring (China)	*a*	*a*	*a*
2 Norin 61 (Japan)	*a*	*a*	*a*
3 Chousen 30 (Korea)	*b*	*a*	*a*
4 Chousen 57 (Korea)	*b*	*a*	*a*
5 T116 (Turkey)	*a*	*a*	*b*
6 K79 (Japan)	*a*	*b*	*a*
7 Nobeokabouzu (Japan)	*a*	*a*	*c*
8 Chinsanwase (China)	*a*	*a*	*d*
9 Gnatruche (USA)	*a*	*c*	*a*
10 Hua Non 9 (China)	*c*	*a*	*a*
11 Hosogara (Japan)	*a*	*a*	*e*
12 Waratah (Australia)	*a*	*d*	*a*

Table 2 *Sgp-3* alleles in wheat cultivars

Cultivar (origin)	Allele[a]		
	Sgp-A3	*Sgp-B3*	*Sgp-D3*
1 Chinese Spring (China)	*a*	*a*	*a*
2 Norin 61 (Japan)	*b*	*a*	*a*
13 Crest (USA)	*a*	*b*	*a*
14 Spica (Australia)	*a*	*c*	*a*
15 Hunter (USA)	*a*	*a*	*a*

[a] Despite having the same genotype, Chinese Spring (1) showed a SGP-3 electrophoretic pattern different from Hunter (15)

end (data not shown). This indicates that the 92-kDa SGP is more acidic than the Wx protein. This spot was not subjected to nulli-tetrasomic analysis.

Electrophoretic analyses suggested that the 100-, 108-, and 115-kDa SGPs can be grouped into one type (SGP-1) encoded by three homoeologous genes. We propose to call these genes *Sgp-B1* (100-kDa SGP), *Sgp-D1* (108-kDa SGP), and *Sgp-A1* (115-kDa SGP), respectively (Fig. 3). Further, the results suggest that the 92-kDa SGP is different from both SGP-1 and the 80-kDa SGP. We therefore propose that the 92-kDa SGP be called SGP-2; the 80-kDa SGP be called SGP-3; and that the three spots of SGP-3 on 2D-PAGE gels be called SGP-A3, -B3 and -D3 (Fig. 3). The presence of three homoeologous genes each for SGP-1 and SGP-3 follows the rule that many genes in common wheat are triplicated due to allohexaploidy involving the AA, BB and DD genomes (Hart 1983).

Relative locations of genes *Sgp-1* and *Sgp-3*

In the deletion lines for chromosome arms 7AS, 7BS and 7DS, 2D-PAGE determined the presence or absence of SGP-1 and SGP-3 (Fig. 4). The results obtained from the deletion lines for 7BS and 7DS did not clearly reveal the relative positions of the two genes *Sgp-1* and *Sgp-3* on the chromosome arms. On the other hand, the gene order on 7AS was suggested by the results from the lines for 7AS. Three of the 7AS deletion lines (7AS-2, -5, and -11) produced SGP-A1 but not SGP-A3, indicating that the *Sgp-A1* gene was closer to the centromere than *Sgp-A3*. In addition, the Wx-A1 protein (coding gene *Wx-A1*, located on 7AS; Chao et al. 1989) was detected only in 7AS-12 but not in the other ten 7AS deletion lines (Yamamori et al. 1994; present results). The observation that SGP-A1 and -A3 were present in 7AS-1 and -9, whereas the Wx-A1 protein was absent, indicates that *Wx-A1* is the most distal among the three genes. Consequently, the gene order on 7AS should be 'centromere–*Sgp-A1*–*Sgp-A3*–*Wx-A1*'.

Two different spot patterns for SGP-D3 deficiency

In lines N7D-T7B and 7DS-1, the patterns of the SGP-3 spots on 2D-PAGE gels were different from those appearing in the other four lines (N7D-T7A and 7DS-2, -4, -5). N7D-T7B (Fig. 2) and 7DS-1 produced electrophoregrams which lacked the SGP-A3 spot. On the other hand, N7D-T7A (Fig. 2) and three deletion lines lost the SGP-D3 spot. All these lines lacked the whole or part of chromosome 7D, on which the coding gene for one of the three SGP-1 spots is located. Therefore, the lines should show identical SGP-3 spots on electrophoresis. Why we observed two different patterns is unknown.

Variations of starch granule proteins in common wheat

SDS-PAGE of 1960 cultivars revealed that a few of them carried variant alleles for SGP-1 (Table 1, Fig. 5 A). Three *Sgp-1* alleles in the standard cultivar, Chinese Spring, were designated *Sgp-A1a*, *-B1a* and *-D1a*. Three cultivars having the null allele *Sgp-A1b* lacked SGP-A1; one Japanese

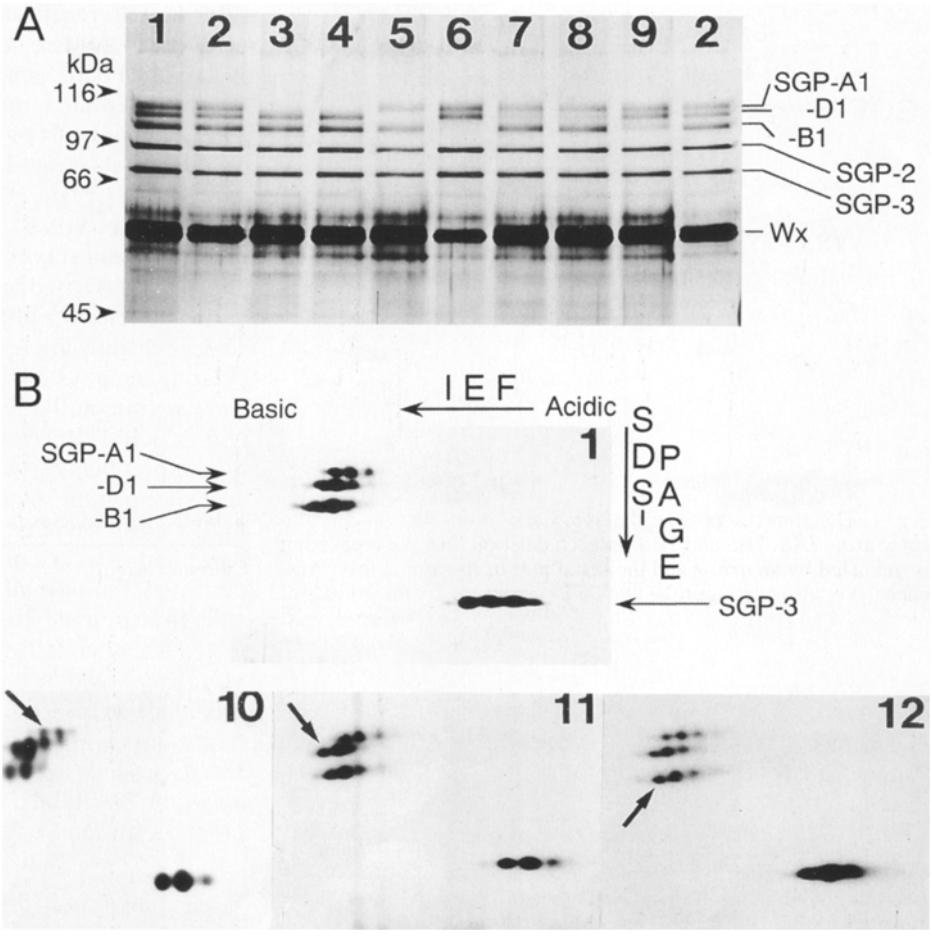

Fig. 5A, B Variation of SGP-1 in wheat cultivars detected by SDS-PAGE (**A**) and 2D-PAGE (**B**). The *numbers* on the gels correspond to the numbers in Table 1. *Arrows* in (**B**) indicate the varied SGP-1

cultivar with *Sgp-B1b* lacked SGP-B1; and one Turkish cultivar with *Sgp-D1b* lacked SGP-D1. *Sgp-B1c* in one American cultivar produced a decreased level of SGP-B1 with a little-more acidic pI than the standard SGP-B1. *Sgp-D1c* in two Japanese cultivars produced a decreased level of SGP-D1 with a normal pI, whereas *Sgp-D1d* in one Chinese cultivar yielded a decreased level of SGP-D1 showing a little-more basic pI than the standard type.

2D-PAGE of 406 cultivars (165 from Japan, 97 from China, 96 from Australia, and 48 from North America) detected pI variations for SGP-A1, -B1 and -D1 (Table 1, Fig. 5 B). A variant allele *Sgp-D1e* in three cultivars produced SGP-D1 with an altered pI (a little-more basic than the standard type in Chinese Spring). *Sgp-A1c* in one Chinese cultivar and *Sgp-B1d* in one Australian cultivar produced altered SGPs showing a little-more acidic pI than Chinese Spring.

2D-PAGE also demonstrated variation or deficiency for the SGP-3 spots (Table 2, Fig. 6). Some cultivars (called *Sgp-A3b*) were considered to lack SGP-A3 because of the presence of the null allele *Sgp-A3*. Another null allele for the SGP-B3 spot (*Sgp-B3b*) occurred in one American cultivar. The allele *Sgp-B3c* in one Australian cultivar produced SGP-B3 with a varied pI (a little-more basic than the standard type). In addition, size variations among three SGP-3 spots were observed (Fig. 6). In 16 cultivars, including Chinese Spring, the sizes of the SGP-A3, -B3 and -D3 spots were almost the same. However, the other cultivars produced spots of different sizes; SGP-D3 was the largest and SGP-A3 the smallest.

Discussion

In rice and maize (diploid cereals), the SDS-PAGE technique used in this study detected three SGP bands which had higher molecular masses than the Wx proteins of these cereals (Fig. 1 A). These three SGPs may correspond to SGP-1, -2 and -3 of common wheat, because, in the three monocots, comparative mapping of genes and/or DNA markers has indicated chromosome homoeology (Ahn et al. 1993; Devos et al. 1994; Kurata et al. 1994), which suggests the presence of orthologous genes.

Schofield and Greenwell (1987) listed five integral SGPs of wheat (59, 77, 86, 95, and 145 kDa), of which the 59-kDa SGP is the Wx protein. Although the modified SDS-PAGE method which we used did not clearly detect a visible band for the 145-kDa SGP, this system seemed to separate the 77-, 86- and 95-kDa SGPs into five protein

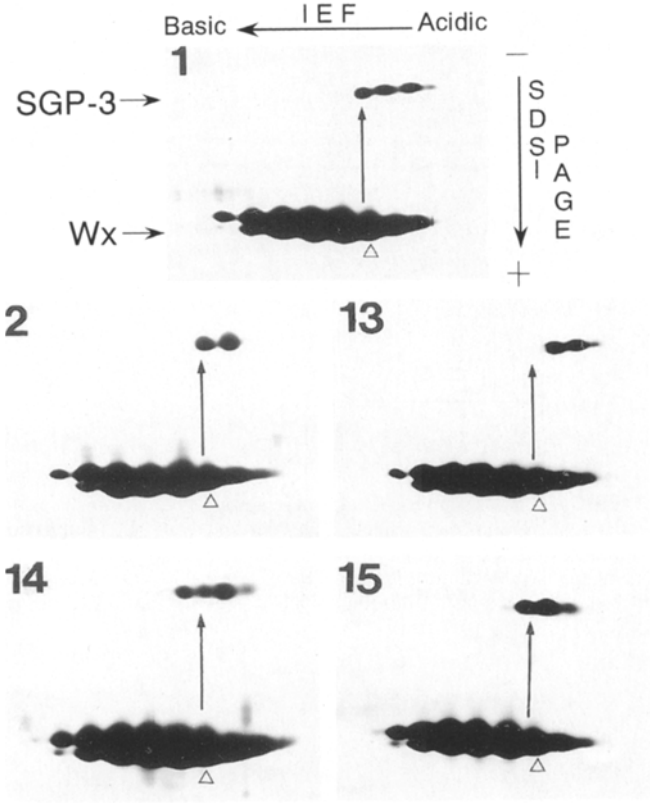

Fig. 6 Variation of SGP-3 in wheat cultivars. *Arrows* indicate the positions of the SGP-B3 spot relative to that of the Wx protein. The *triangles* (Δ) indicate the same positions of the Wx-B1 protein. The *numbers* on 2D-PAGE gels correspond to those in Table 2

bands (Fig. 1). Besides the Wx protein, Denyer et al. (1995) reported five starch granule-bound proteins (77–105 kDa) in wheat endosperm which were fractionated by SDS-PAGE. They showed that these five proteins (77, 90 and 100–105 kDa) were associated with activities of starch synthase or starch branching enzyme, and three of them (100–105 kDa proteins) were regarded as structurally related products of three homoeologous genes. The five 77–105-kDa proteins are likely to be identical to the five SGP bands detected in our SDS-PAGE analysis since both studies used starches washed with SDS-containing solution, employed almost the same procedures for protein extraction, and provided analogous electrophoregrams on SDS-PAGE. Moreover, our mapping of *Sgp-1* loci (*Sgp-A1*, *-B1* and *-D1*) on the group-7 chromosomes is consistent with the chromosomal locations of the genes for the three 100–105-kDa proteins determined by Denyer et al. (1995).

Deletion mapping suggested that the order of the three genes on chromosome arm 7AS was 'centromere–*Sgp-A1*–*Sgp-A3*–*Wx-A1*'. Hohmann et al. (1994) described an almost identical linear order of molecular markers on the short arms of the group-7 chromosomes, although they assumed the presence of an interstitial inversion on 7AS or 7DS of Chinese Spring. Possibly, the order of the three genes on 7AS may also be conserved on 7DS and 7BS. The deletion line 7DS-4 produced SGP-D1 but not SGP-D3 (Fig. 4). This result suggests that the *Sgp-D1* gene is closer to the centromere than *Sgp-D3*. Aneuploid analyses showed that both *Sgp-B1* and *Sgp-B3* were located on 7BS. On the other hand, the *Wx-B1* gene is located on 4AL, to which a distal part of 7BS has been translocated (Chao et al. 1989; Liu et al. 1992). A separation of *Wx-B1* from *Sgp-B1* and *-B3* on 7BS would occur since *Wx-B1* is more terminal than *Sgp-B1* and *Sgp-B3*.

The present survey of wheat germ plasm found five null alleles (*Sgp-A1b*, *-B1b*, *-D1b*, *Sgp-A3b* and *-B3b*) and seven variant alleles yielding an altered SGP-1 and SGP-B3. The three null alleles for SGP-1 will be useful for elucidating the physiological function of SGP-1. In a cultivar carrying one null allele, e.g. *Sgp-A1b*, the phenotypic effect of SGP-A1 deficiency will be masked to some extent by the products of two alleles, *Sgp-B1a* and *-D1a*. Thus, an experimental material which does not produce three SGP-1 classes will be necessary. In the case of the Wx protein, the discovery of three null alleles allowed us to genetically eliminate all three Wx proteins (Wx-A1, -B1 and -D1) from common wheat through cross breeding. The selected wheat yielding no Wx proteins showed a waxy (amylose-free) endosperm starch (Nakamura et al. 1995; Yamamori et al. 1995). Similarly, crossing among the cultivars carrying different null *Sgp-1* alleles will, if it is not lethal, produce a variant wheat having no SGP-1. In such a variant, it will be particularly interesting to examine the properties of endosperm starch.

References

Ahn S, Anderson JA, Sorrells ME, Tanksley SD (1993) Homoeologous relationships of rice, wheat and maize chromosomes. Mol Gen Genet 241:483–490

Ainsworth C, Clark J, Balsdon J (1993) Expression, organization and structure of the genes encoding the *waxy* protein (granule-bound starch synthase) in wheat. Plant Mol Biol 22:67–82

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

Denyer K, Sidebottom C, Hylton CM, Smith AM (1993) Soluble isoforms of starch synthase and starch-branching enzyme also occur within starch granules in developing pea embryos. Plant J 4:191–198

Denyer K, Hylton CM, Jenner CF, Smith AM (1995) Identification of multiple isoforms of soluble and granule-bound starch synthase in developing wheat endosperm. Planta 196:256–265

Devos KM, Chao S, Li QY, Simonetti MC, Gale MD (1994) Relationship between chromosome 9 of maize and wheat homoeologous group-7 chromosomes. Genetics 138:1287–1292

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:Li ZS, Xin ZY (eds) Proc 8th Int Wheat Genet Symp, China Agri Scientech Press, Beijing, pp 211–216

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

Goldner WR, Boyer CD (1989) Starch granule-bound proteins and polypeptides: the influence of the *waxy* mutations. Starch/stärke 41:250–254

Greenwell P, Schofield JD (1986) A starch granule protein associated with endosperm softness in wheat. Cereal Chem 63:379–380

Hart GE (1983) Hexaploid wheat (*Triticum aestivum* L. em Thell). In: Tanksley SD, Orton TJ (eds) Isozymes in plant genetics and breeding, Part B. Elsevier Science Publishers B.V., Amsterdam, pp 35–56

Hohmann U, Endo TR, Gill KS, Gill BS (1994) Comparison of genetic and physical maps of group-7 chromosomes from *Triticum aestivum* L. Mol Gen Genet 245:644–653

Kagawa H, Hirano H, Kikuchi F (1988) Variation of glutelin seed storage protein in rice (*Oryza sativa* L.). Jpn J Breed 38:327–332

Kurata N, Moore G, Nagamura Y, Foote T, Yano M, Minobe Y, Gale MD (1994) Conservation of genome structure between rice and wheat. Bio/ Technology 12:276–278

Liu CJ, Atkinson MD, Chinoy CN, Devos KM, Gale MD (1992) Nonhomoeologous translocations between group-4, -5 and -7 chromosomes within wheat and rye. Theor Appl Genet 83:305–312

Lowy GDA, Sargeant JG, Schofield JD (1981) Wheat starch granule protein: the isolation and characterization of a salt-extractable protein from starch granules. J Sci Food Agric 32:371–377

Mu C, Harn C., Ko YT, Singletary GW, Keeling PL, Wasserman BP (1994) Association of a 76-kDa polypeptide with soluble starch synthase I-activity in maize (cv B73) endosperm. Plant J 6:151–159

Nakamura T, Yamamori M, Hirano H, Hidaka S (1993 a) The waxy (Wx) proteins of maize, rice and barley. Phytochemistry 33:749–753

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

Nakamura T, Yamamori M, Hirano H, Hidaka S, Nagamine T (1995) Production of waxy (amylose free) wheats. Mol Gen Genet 248:253–259

Preiss J (1991) Biology and molecular biology of starch synthesis and its regulation. Oxford Surveys Plant Mol Cell Biol 7:59–114

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

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

Sears ER (1966) Nullisomic-tetrasomic combinations in hexaploid wheat. In: Riley R, Lewis KR (eds) Chromosome manipulation and plant genetics. Oliver and Boyd, Edinburgh, pp 29–45

Sears ER, Sears LMS (1978) The telocentric chromosomes of common wheat. In: Ramanujam S (ed) Proc 5th Int Wheat Genet Symp. Indian Soc Genet Plant Breed, New Delhi, pp 389–407

Skerritt JH, Frend AJ, Robson LG, Greenwell P (1990) Immunological homologies between wheat gluten and starch granule proteins. J Cereal Sci 12:123–136

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

Yamamori M, Nakamura T, Endo TR, Nagamine T (1994) Waxy protein deficiency and chromosomal location of coding genes in common wheat. Theor Appl Genet 89:179–184

Yamamori M, Nakamura T, Nagamine T (1995) Inheritance of waxy endosperm character in a common wheat lacking three Wx proteins. Breed Sci 45:377–379