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Evidence from principal component analysis for improvement of grain shape- and spikelet morphology-related traits after hexaploid wheat speciation

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Grain shape and size are involved in the main components of the domestication syndrome in cereals. Wheat grain shape has been dramatically altered at each stage of the domestication of tetraploid wheat and through common wheat speciation. To elucidate the evolutionary change of wheat grain shape, principal component (PC) analysis of grain shape-related traits was first conducted using wild and cultivated tetraploid, synthetic hexaploid, and common wheat accessions. The synthetic hexaploid wheat lines were previously produced through interspecific crosses between two common wheat progenitors, tetraploid wheat and *Aegilops tauschii*, and produced grains similar to those of cultivated tetraploid wheat. To identify genetic loci related to the difference in grain shape between common wheat and the synthetic wheat, the 15 traits related to grain and spikelet shape were measured in 108 F_2 individuals between Norin 61 and a synthetic wheat line, and the first three PC values for the 15 traits, PC1, PC2 and PC3, were mapped as quantitative traits in the F_2 population. In total, six QTLs, found on chromosomes 1A, 5A, 1D, 2D and 7D, showed significant LOD scores. Among them, a QTL for PC2, located on the 2DS chromosomal region near the *Ppd-D1* locus, mainly contributed to the phenotypic difference in grain shape. *Tg-D1*, controlling tenacious glume phenotype, was located at a similar region to the 2DS QTL, which suggested that the *Tg-D1* locus pleiotropically affects not only glume toughness but also spikelet and grain shape in hexaploid wheat. Therefore, it was predicted that wheat grains were rapidly improved toward a shorter and rounder phenotype accompanied with free-threshing wheat formation.

Key words: allopolyploidization, grain shape, quantitative trait locus, synthetic wheat, tenacious glume

INTRODUCTION

An increase in grain size, significantly related to grain yield, is considered to be one characteristic of the domestication syndrome (Fuller, 2007). In rice, grain shape is recognized as a major target for artificial selection during the domestication process, as is grain size (Kovach et al., 2007). In contrast to rice, wheat grain shape is not considered to have been a component of the domestication syndrome, although abundant variation in grain shape has been found in wheat and its relatives (Gegas et al., 2010). In addition, grain size and shape are associated with milling quality and yield in wheat (Evers et al., 1990; Breseghello and Sorrells, 2006). Therefore, grain size and shape have been two of the main targets for artificial selection and breeding in common wheat as well as in other cereals.

Many quantitative trait loci (QTLs) for grain size and shape have been identified in common wheat, *Triticum aestivum* L. (Campbell et al., 1999; Dholakia et al., 2003; Breseghello and Sorrells, 2006, 2007; Sun et al., 2009; Gegas et al., 2010). Wheat domestication occurred in einkorn and tetraploid wheat (Salamini et al., 2002; Matsuoka, 2011), and a relatively small grain with a long, thin shape was improved to a uniform larger grain with

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a short, wide shape during domestication (Gegas et al., 2010). In fact, wheat grain size predominantly increased early in domestication (Gegas et al., 2010). There is little information on improvement of grain shape during the process of common wheat speciation, though in a mapping population with recombinant inbred lines between a synthetic hexaploid wheat line, W7984, and a common wheat cultivar, Opata 85, the strongest QTL for kernel length was on chromosome 5B (Breseghello and Sorrells, 2007). Synthetic hexaploid wheat can be artificially produced from crosses between two common wheat progenitors, *Triticum turgidum* L. and *Aegilops tauschii* Coss. (Matsuoka and Nasuda, 2004). Therefore, synthetic hexaploid wheat is useful in elucidating the evolutionary alteration in grain size and shape that occurred early in common wheat breeding. Primitive hexaploid wheat species exhibit broad variation in grain size and shape, in contrast to modern wheat varieties, meaning that the modern breeding germplasm has lost grain shape variation, probably due to selection for more uniform grain shape in the elite varieties (Gegas et al., 2010). In the elite wheat varieties, grain width is markedly increased, whereas grain length is decreased (Gegas et al., 2010).

In our previous studies, a number of synthetic hexaploid wheat lines were produced from crosses between *T. turgidum* cultivar Langdon (Ldn) and various *Ae. tauschii* accessions (Takumi et al., 2009a; Kajimura et al., 2011). Glumes of the synthetic hexaploid wheat lines tenaciously enclose grains, which makes it difficult to thresh seeds easily from spikelets. In addition, the wheat synthetics seem to provide larger grains than the elite varieties. The hulled trait is generally controlled by the *Tg* (tenacious glume) and *q* (speltoid) loci in wheat (Salamini et al., 2002; Matsuoka, 2011; Dvorak et al., 2012). Recent reports demonstrated that spikelet hull and panicle traits affect grain morphology in rice (Song et al., 2007; Shomura et al., 2008; Huang et al., 2009; Zhou et al., 2009). In wheat, the relation between spikelet morphology and grain shape is not clear. Because of large variations in spikelet and floral morphological traits in the *Ae. tauschii* population (Matsuoka et al., 2009; Takumi et al., 2009b), synthetic wheat lines can be used to estimate the effects of spikelet morphology on grain shape in a hexaploid genetic background.

Here, to elucidate improvement of grain shape after the birth of common wheat, we compared grain shape-related traits between common wheat cultivars and synthetic hexaploid wheat lines. Principal component (PC) analysis can be used for examining the main patterns of variation in the morphological data (Klingenberg, 2010). In fact, QTL analysis based on PC values for leaf morphology was quite useful for understanding natural variations in leaf shape and size of *Antirrhinum* species (Langlade et al., 2005). Together with the phenotypic variation in grain shape among several wheat groups, we discuss the relationship between the identified QTLs for PC values of grain shape-related traits and wheat speciation.

MATERIALS AND METHODS

Plant materials In total, 26 *Ae. tauschii* accessions, 26 tetraploid wheat accessions, 22 common wheat cultivars, and 34 synthetic hexaploid wheat lines were used in this study (Table 1). The tetraploid wheat included two wild species, *Triticum dicoccoides* and *Triticum araraticum*, and seven subspecies of a cultivated *T. turgidum*. The common wheat cultivars included 9 Japanese cultivars and 9 KU-numbered landraces from Afghanistan, Nepal, Iran, and the United Kingdom. For production of the wheat synthetics, the tetraploid wheat cultivar Langdon (Ldn) was used as the female parent and was crossed with each of the 26 *Ae. tauschii* accessions. The F_1 progeny were grown and selfed to produce synthetics (herein designated the F_2 generation) as previously reported (Takumi et al., 2009a; Mizuno et al., 2010). All 30 synthetics (Ldn x *Ae. tauschii*) were independently generated through unreduced gamete formation in each of the triploid F_1 hybrids (Matsuoka and Nasuda, 2004). The synthetics thus contained the A and B genomes from Ldn and the diverse D genomes originating from the *Ae. tauschii* pollen parents. Some of the triploid F_1 hybrids between Ldn and *Ae. tauschii* show abnormal growth, such as hybrid necrosis, hybrid chlorosis and severe growth abortion (Matsuoka et al., 2007; Mizuno et al., 2010). Hybrids showing necrosis, chlorosis and severely aborted growth were excluded from the 27 synthetics. In addition, four synthetic wheat lines were newly established through crossing a *Triticum durum* cultivar (KU-136) and one wild wheat (*Triticum dicoccoides*) accession (KU-8736A) with the four *Ae. tauschii* accessions, KU-2124, AE1038, PI476874 and KU-2025, in the present study (Table 1). The four synthetic lines exhibited normal plant architecture. The somatic chromosome number of 42 was previously confirmed using two F_3 seeds from one F_2 plant of each synthetic (Kajimura et al., 2011). In the present study, F_3 plants derived from one F_2 plant of each synthetic were used.

For a mapping population, 108 F_2 individuals from a single F_1 plant between a Japanese common wheat cultivar, Norin 61, and a synthetic hexaploid wheat line between Ldn and *Ae. tauschii* PI476874 were used.

Evaluation of grain shape and other morphological traits Seeds of the sample accessions were sown in November 2009 (three seeds per accession) and plants grown under field conditions at Kobe University. The accessions were arranged in the field using a randomized design. For each accession, a single healthy individual was chosen for analysis of grain-shape variation. Four traits, seed length, seed width, seed height and the

Table 1. Strain numbers and sources of wheat accessions used in this study

Ae. tauschii accessions (n = 26)	
	AE1090, IG47259, KU-2069, KU-2091, KU-2093, KU-2097, KU-2103, KU-2126, PI476874, KU-2811, KU-2124, AE929, IG126387, IG48042, KU-20-8, KU-2092, KU-2105, KU-2158, KU-2159, KU-2829A, KU-2814, KU-2090, KU-2152, IG46663, IG131606, CGN10768
Tetraploid wheat accessions (n = 26)***	
	T. timopheevii subsp. *armeniacum*: KU-8451, KU-8672, KU-8713, KU-8944
	T. turgidum subsp. *dicoccoides*: KU-8736A, KU-1952, KU-8941, KU-14401, KU-8817, KU-1978B, KU-1959B
	T. turgidum subsp. *dicoccon*: KU-7309, KU-1063a, KU-10501, KU-3371, KU-10497
	T. turgidum subsp. *durum*: KU-136, KU-328, KU-125, Langdon (Ldn)
	T. turgidum subsp. *pyramidale*: KU-9882
	T. turgidum subsp. *polonicum*: KU-9894, KU-144
	T. turgidum subsp. *paleocolchicum*: KU-190-2
	T. orientale Perc.: KU-3368
	T. abysinicum Vav.: KU-9281
Common wheat cultivars (n = 22)	
	Akadaruma, Fukuwase-komugi, Shirasagi-komugi, Norin 61, Igachikugo, Haya-komugi, Aka-komugi, Akabozu, Kinuiroha, Chinese Spring, Bobwhite, Mironovskaya 808, Fielder, KU-330, KU-370, KU-3045, KU-3083, KU-3121, KU-3126, KU-3184, KU-3189, KU-4770
Synthetic wheat lines (n = 34)	
	Ldn×AE1090, Ldn×IG47259,* Ldn×KU-2069, Ldn×KU-2091, Ldn×KU-2093, Ldn×KU-2097, Ldn×KU-2103,* Ldn×KU-2126, Ldn×PI476874,* Ldn×KU-2811, Ldn×KU-2124,* Ldn×AE929, Ldn×IG126387, Ldn×IG48042, Ldn×KU-20-8, Ldn×KU-2092, Ldn×KU-2105, Ldn×KU-2158, Ldn×KU-2159, Ldn×KU-2829A, Ldn×KU-2814, Ldn×KU-2090, Ldn×KU-2152, Ldn×IG46663, Ldn×IG131606, Ldn×CGN10768, KU-136×KU-2124, KU-8736A×AE1038**, KU-8736A×PI476874, KU-8736A×KU-2025

KU: Plant Germ-Plasm Institute, Graduate School of Agriculture, Kyoto University, Japan
PI: National Small Grains Research Facility, USDA-ARS, USA
IG: International Center for Agricultural Research in the Dry Areas (ICARDA)
CGN: Centre for Genetic Resources, The Netherlands
AE: Institut für Pflanzengenetil und Kulturpflanzenforschung (IPK), Germany
*: Two synthetic lines produced from independent crosses
**: An accession of *Ae. tauschii*
***: Taxonomic classification of tetraploid wheat was referred to Matsuoka (2011), catalogue of KU, and the Wheat Classification Table Site (http://www.k-state.edu/wgrc/Taxonomy/taxintro.html).

length-to-width ratio, were used for evaluation of grain shape. The grain shape measurements were done using more than 10 seeds for each accession.

In the F_2 mapping population, the four traits for grain shape and the following eleven traits were assessed: spike length (SL), spikelet length (SpL), empty glume length (GL), empty glume width (GW), lemma length (LL), lemma width (LW), number of spikelets per spike (SpN), number of seeds per spike, hundred seed weight (HSW), spikelet density (SpD), and selfed seed fertility (SSF). Traits were measured on the central spikelets of the first, second, and third spikes before anthesis. For each spikelet, only the components of the first and second florets were used; two lemmata and two empty glumes were measured. The mean of the replicated measurements over three spikes was calculated for each trait and used as the trait value in subsequent analyses.

The data were statistically analyzed using JMP software ver. 5.1.2 (SAS Institute, Cary, NC, USA). The correlations among the examined traits were estimated based on Pearson's correlation coefficient values. PC analysis was also conducted using JMP software.

Genotyping and construction of a linkage map

To amplify PCR fragments of simple sequence repeat (SSR) markers, total DNA was extracted from the parents and F_2 individuals using standard procedures. For the SSR genotyping, 40 cycles of PCR were performed using 2x Quick Taq HS DyeMix (TOYOBO, Osaka, Japan) and the following conditions: 10 s at 94°C, 30 s at the annealing temperature, and 30 s at 68°C. The last step was incubation for 1 min at 68°C. Information on SSR markers and their annealing temperature was obtained from the National BioResource Project (NBRP) KOMUGI web site (http://www.shigen.nig.ac.jp/wheat/komugi/strains/aboutNbrpMarker.jsp) and the GrainGenes web site (http://wheat.pw.usda.gov/GG2/maps.shtml). The PCR products were separated by 2% agarose or 13% nondena-

turing polyacrylamide gels and visualized under UV light after staining with ethidium bromide. For polyacrylamide gel electrophoresis, the high efficiency genome scanning system (Nippon Eido, Tokyo, Japan) of Hori et al. (2003) was used. Genetic mapping was performed using MAPMAKER/EXP version 3.0b software (Lander et al., 1987). The threshold for log-likelihood scores was set at 3.0, and genetic distances were calculated with the Kosambi function (Kosambi, 1944). Chromosomal assignment of SSR markers was generally conducted based on other reference maps (Somers et al., 2004; Torada et al., 2006; Kobayashi et al., 2010a).

Polymorphism at the *Ppd-D1* locus was detected using allele-specific primers according to Beales et al. (2007). A common forward primer, Ppd-D1_F (5'-ACGCCTCCCACTACACTG-3'), and two reverse primers, Ppd-D1_R1 (5'-GTTGGTTCAAACAGAGAGC-3') and Ppd-D1_R2 (5'-CACTGGTGGTAGCTGAGATT-3'), were used for this PCR analysis. PCR products amplified with the Ppd-D1_F and Ppd-D1_R2 detected a 2,089-bp deletion in the 5' upstream region of *Ppd-D1*, indicative of the photoperiod-insensitive *Ppd-D1a* allele (Beales et al., 2007). The amplified PCR products were separated by electrophoresis through a 2% agarose gel and stained with ethidium bromide.

The genotype at the *q* locus was also confirmed using restriction site polymorphisms as previously reported (Asakura et al., 2009). Three intragenic regions were amplified using *q*-specific primers, and then the amplified products were digested with *Msp*I, *Taq*I or *Mbo*I. After separation of the digested fragments by electrophoresis through a 2% agarose gel and staining with ethidium bromide, the presence or absence of the three restriction sites was determined with reference to the previous data (Asakura et al., 2009).

QTL analysis of morphological traits QTL analysis was carried out by composite interval mapping with Windows QTL Cartographer ver. 2.5 software (Wang et al., 2007) using the forward and backward method. A log-likelihood (LOD) score threshold of 3.0 was determined. The percentage of phenotypic variation explained by a QTL for a trait and any additive effects were also estimated using the software.

Scanning electron microscope observation of wheat grains Grains were snapped in the middle using a straight razor, and a transverse section was analyzed by an S-3400N scanning electron microscope (Hitachi High-Technology, Tokyo, Japan). Scanning electron microscopy was conducted without any pretreatment at an accelerating voltage of 8.00 kV under low vacuum conditions of 70 Pa at –25°C according to previous reports (Araki et al., 2009; Kobayashi et al., 2010b).

RESULTS

Grain shape variation in various wheat groups Four traits associated with grain shape were compared among five wheat groups, i.e., wild diploid wheat (*Ae. tauschii*), wild tetraploid wheat, cultivated tetraploid wheat, common wheat, and synthetic hexaploids (Table 2). In all four traits, significant differences ($P < 0.05$) were observed among the five groups. The *Ae. tauschii* grains were the smallest of the five groups, and the wild wheat groups showed narrower grains than cultivated ones. The wild tetraploid wheat had the slenderest grains, and grain width and height were much larger in the cultivated groups than in the wild groups.

PC analysis based on the four traits was performed in all accessions of the four allopolyploid wheat groups. The first two values for the four traits, PC1 and PC2, were mainly related to grain shape and size (Table 3). The accumulated proportion of PC1 and PC2 in the total variation of grain shape was 96.9% (75.1% for PC1 and 21.8% for PC2). Scatter plots with PC1 and PC2 of the allopolyploid wheat were continuous, but with three subgroups (Fig. 1). The 11 wild tetraploid wheat accessions formed one subgroup, and the 22 common wheat cultivars belonged to distinct subgroup. The distribution of the cultivated tetraploid wheat accessions and synthetic hexaploid wheat lines overlapped.

Thirty hexaploid synthetic lines were independently derived from interspecific crosses between Ldn and 27 parental *Ae. tauschii* accessions. These synthetics were used for comparison of transmission of D-genome varia-

Table 2. Grain shape characters in the five wheat groups examined in this study

	Seed length			Seed width			Seed height			Length/width ratio		
	mean ± SD	min	max	mean ± SD	min	max	mean ± SD	min	max	mean ± SD	min	max
Ae. tauschii (n = 26)	5.36 ± 0.53^{e}	4.44	6.35	2.46 ± 0.34^{b}	2.03	3.13	1.52 ± 0.27^{c}	1.06	1.93	2.20 ± 0.28^{c}	1.67	2.71
Wild 4x wheat (n = 11)	9.86 ± 0.38^{a}	9.21	10.35	2.06 ± 0.19^{c}	1.82	2.51	2.27 ± 0.16^{b}	2.01	2.52	4.81 ± 0.35^{a}	4.05	5.19
Cultivated 4x wheat (n = 15)	8.52 ± 0.78^{c}	7.22	10.19	3.06 ± 0.32^{a}	2.38	3.46	3.20 ± 0.28^{a}	2.65	3.59	2.81 ± 0.34^{b}	2.27	3.29
Common wheat (n = 22)	6.92 ± 0.42^{d}	6.25	7.61	3.15 ± 0.29^{a}	2.29	3.73	3.09 ± 0.28^{a}	2.45	3.74	2.21 ± 0.22^{c}	1.90	2.82
Synthetic wheat (n = 34)	9.03 ± 0.47^{b}	8.08	10.01	3.07 ± 0.27^{a}	2.40	3.50	3.17 ± 0.32^{a}	2.39	3.83	2.97 ± 0.36^{b}	2.47	4.00

Mean values with the same letters were not significantly different ($P > 0.05$) (Tukey-Kramer HSD test).

Table 3. Eigenvectors for PC1 and PC2 based on the PC analysis of grain shape in tetraploid, common and synthetic hexaploid wheat

Trait	PC1	PC2
Eigenvalue	3.00	0.87
Contribution rate (%)	75.1	21.8
Total contribution rate (%)	75.1	96.9
Seed length	–0.38	0.80
Seed width	0.54	0.29
Seed height	0.49	0.50
Length/width of seed	–0.56	0.18

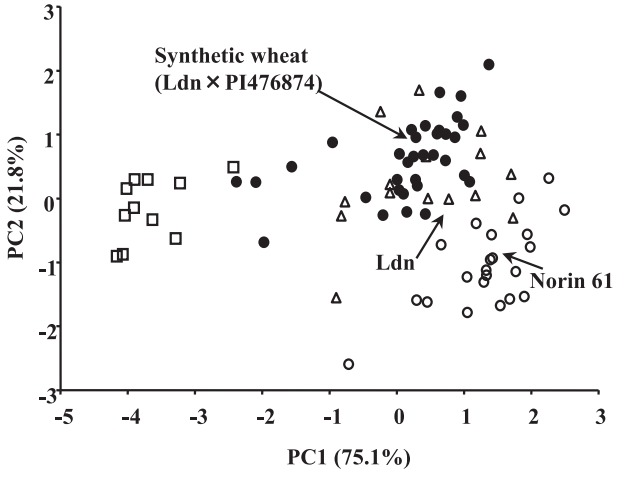

Fig. 1. Scatter plots based on the two PC values of grain shape in wild tetraploid (white squares), cultivated tetraploid (white triangles), synthetic hexaploid (black circles), and common (white circles) wheat. Arrows indicate positions of the two parental lines of the mapping population and Ldn.

tion in the four traits from the parental *Ae. tauschii* accessions to the hexaploid wheat background. Scatter plots of the four traits showed significant correlations between the diploid and hexaploid wheat backgrounds (Fig. 2). Therefore, the variation in grain shape in *Ae. tauschii* was well maintained in the hexaploid background of the synthetic wheat lines.

Construction of a linkage map and QTL analysis of grain shape A total of 842 SSR primer sets were tested for polymorphisms between Norin 61 and a synthetic line (Ldn x PI476874), and 243 sets (28.9%) showed polymorphism for one or two alleles. In addition, the *Ppd-D1* locus on chromosome 2D was mapped using allele-specific primer sets (Beales et al., 2007). Norin 61 contained a photoperiod-insensitive allele at *Ppd-D1* and the synthetic wheat line contained a sensitive allele. Similarly, genotypes of the *q* locus on chromosome 5A were compared between the mapping parents using allele-specific restriction sites (Asakura et al., 2009), but no polymorphisms were found. This indicated that Norin 61 and

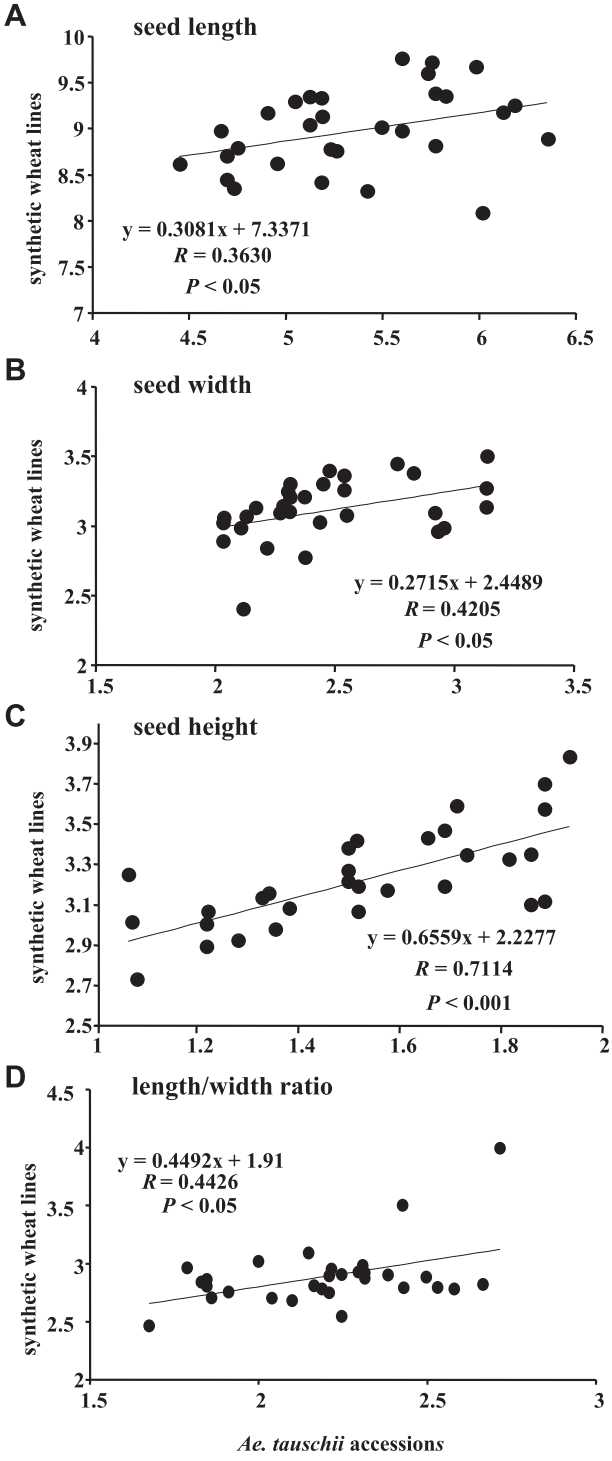

Fig. 2. Correlation of the four grain shape-related traits, seed length (A), seed width (B), seed height (C) and length-to-width ratio (D), between the synthetic wheat lines and *Ae. tauschii* accessions.

the synthetic wheat line share the same genotype (a dominant *Q* allele) at the *q* locus. Moreover, a phenotypic marker for non-glaucousness was mapped, and the marker was the well-known *Iw2* on chromosome 2D

(Tsunewaki and Ebana, 1999; Liu et al., 2007). Norin 61 exhibited wax production on the peduncle surface, whereas the synthetic wheat line lacked a waxy appearance. A genetic map was constructed based on the segregation of the 216 loci using the 108 F_2 individuals. The total map length was 3,173.8 cM with an average spacing of 14.6 cM between markers.

The 15 traits related to grain and spikelet shape were measured in the 108 F_2 individuals of the mapping population. The first three PC values for the 15 traits, PC1, PC2 and PC3, were extracted based on the grain and spikelet shape-related traits (Table 4). The accumulated proportion of PC1, PC2 and PC3 in the total variation was 69.3% (30.3% for PC1, 22.7% for PC2, and 16.3% for PC3). PC1 was mainly related to grain, spikelet and spike size, whereas PC2 was associated with grain and spikelet shape. The number of spikelets and seed fertility affected the PC3 value, and effects of PC3 on grain and spikelet morphology were limited.

To identify genetic loci controlling the difference in grain shape between common wheat and synthetic hexaploids, QTLs for PC1, PC2 and PC3 were detected based on the PC values and the genetic map of the F_2 individuals. Six QTLs found on chromosomes 1A, 5A,

Table 4. Eigenvectors for PC1, PC2 and PC3 based on the PC analysis of grain and spikelet shape-related traits in the mapping population

Trait	PC1	PC2	PC3
Eigenvalue	4.55	3.41	2.45
Contribution rate (%)	30.3	22.7	16.3
Total contribution rate (%)	30.3	53.0	69.3
Seed length (L)	0.20	0.32	–0.22
Seed width (W)	0.31	–0.33	0
Seed height (H)	0.18	–0.29	–0.27
Length/width of seed (L/W)	–0.11	0.45	–0.13
Empty glume length (GL)	0.29	0.24	–0.09
Empty glume width (GW)	0.37	–0.17	0.06
Lemma length (LL)	0.33	0.26	–0.01
Lemma width (LW)	0.37	–0.17	0.08
Spikelet length (SpL)	0.35	0.19	0.04
Spike length (SL)	0.17	0.38	0.21
Number of spikelets per a spike (NSp)	0	0.22	0.40
Spikelet density (SpD)	0.21	0.25	–0.18
Grain number per a spike (GN)	0.14	–0.06	–0.18
Hundred grain weight (HGW)	0.37	–0.16	–0.16
Selfed seed fertility (SSF)	0.05	–0.04	0.52

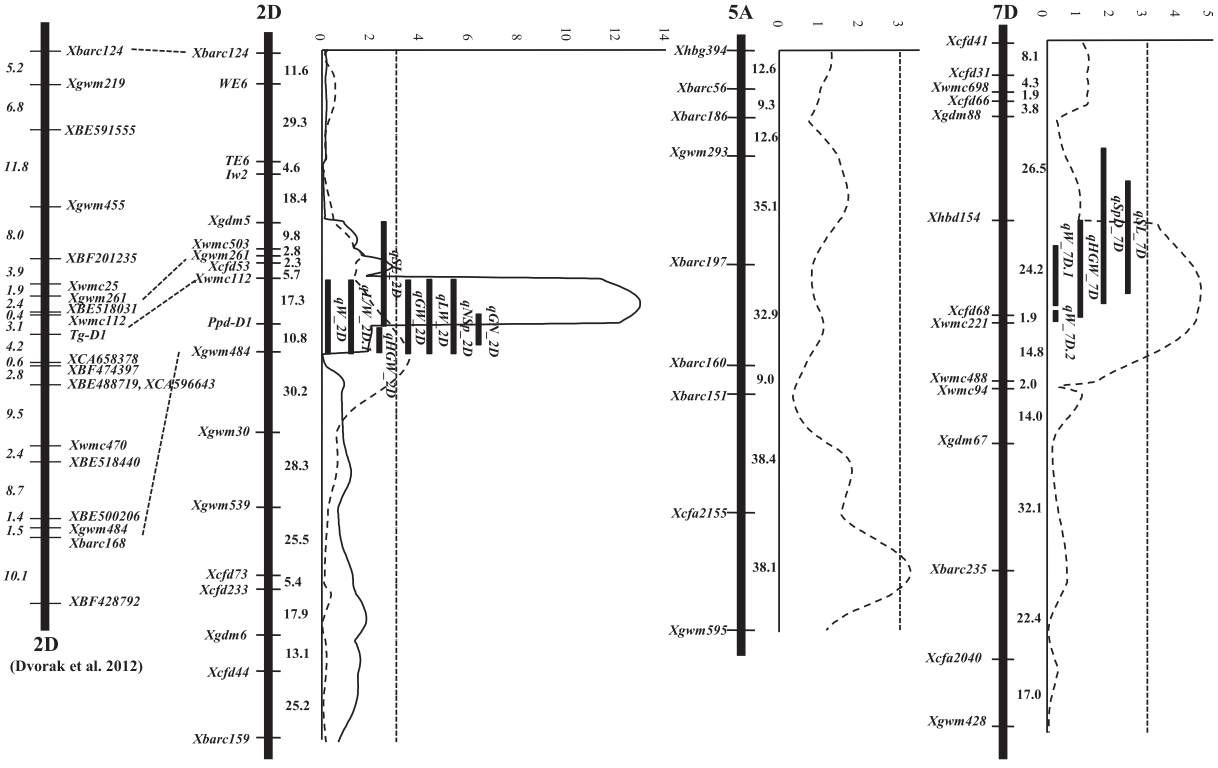

Fig. 3. Linkage maps and QTL-likelihood curves of LOD scores based on the PC1 (broken lines) and PC2 (black lines) values for grain and spikelet shape on chromosomes 5A, 2D and 7D. Black bars at the PC1 and PC2 QTL regions on chromosomes 2D and 7D indicate the QTL regions of each trait examined in the present study. Comparative maps of the 2DS QTL for PC2 and the *Tg-D1* region on chromosome 2D are represented, and the *Tg-D1* position is indicated on chromosome 2D of the DSAt5403 (CS2D) x CS map (Dvorak et al., 2012). Genetic distances are represented in centimorgans on the right of each chromosome. The 3.0 LOD score threshold is indicated by a dashed line.

1D, 2D and 7D showed significant LOD scores > 3.0 ($P < 0.05$) (Fig. 3). A major QTL for PC2 with an LOD score of > 11.2 was located on the short arm of chromosome 2D and contributed 31–41% of the PC2 variation (Table 5). The molecular markers *Xwmc112* and *Ppd-D1* flanked this QTL at an interval of 17.3 cM (Fig. 3). The F_2 individuals carrying the Norin 61 allele at the 2D QTL produced shorter and rounder grains than those with the synthetic wheat allele. No other QTLs for PC2 with an LOD score > 3.0 were detected. Three QTLs for PC1 on chromosomes 5A, 2D and 7D respectively accounted for 22–23%, 10–12% and 11–20% of the phenotypic variation. The 2D QTL for PC1 was located near the QTL for PC2. The F_2 individuals carrying the Norin 61 allele at the 5A or 7D QTLs produced smaller grains than those with the synthetic wheat allele. Two QTLs on chromosomes 1A and 1D contributed 12–14% and 10–13% of the PC3 variation, respectively.

Correlation between grain shape and spikelet shape-related traits To analyze the relationship between grain shape and spikelet shape, correlation coefficients were calculated among the examined traits in the F_2 mapping population (Supplementary Table S1). Seed length showed a significantly positive correlation to length of the empty glume, lemma, spikelet and spike. Seed width was highly correlated to width of the empty glume, lemma and seed height. Significantly high correlations were also found between grain weight and grain shape. These observations indicated that spikelet shape-related traits significantly affect wheat grain shape.

In total, 47 QTLs for grain and spikelet shape-related traits were found based on the morphological data and the genetic map of the F_2 individuals (Supplementary Fig. S1). These QTLs showed significant LOD scores > 3.0 ($P < 0.05$), and were distributed on various wheat chromosomes (Supplementary Table S2). All four traits for grain shape were under the control of chromosome 2D, and QTLs for the seed width and length-to-width ratio were positioned at a similar chromosomal region to that of the 2D QTL for PC2 (Fig. 3). The two QTLs on chromosome 2D explained 17.6–36% of the variation in seed width and 25.3–40.9% in length-to-width ratio. At this region, six other QTLs for empty glume width, lemma width, spike length, number of spikelets, grain number and grain weight also overlapped. These six QTLs provided high LOD scores, and contributed highly to the phe-

Table 5. Summary of the QTLs for three PCs in the mapping population

	Chr.	LOD score	Marker interval	Additive effect	Contribution (%)
PC1	5A	3.02–3.26	*Xcfa2155 - Xgwm595*	–0.85 – –0.80	22 – 23
PC1	2D	3.02–3.57	*Ppd-D1 - Xgwm30*	0.88 – 0.98	10 – 12
PC1	7D	3.08-4.62	*Xhbd154 - Xwmc488*	–1.39 – –0.96	11 – 20
PC2	2D	11.2–12.9	*Xwmc112 - Ppd-D1*	–1.69 – –1.46	31 – 44
PC3	1A	3.00–3.19	*Xbarc119 - Xgwm11*	0.75 – 0.81	12 – 14
PC3	1D	3.05–3.38	*Xcfd92 - Xcfd83*	0.67 – 0.77	10 – 13

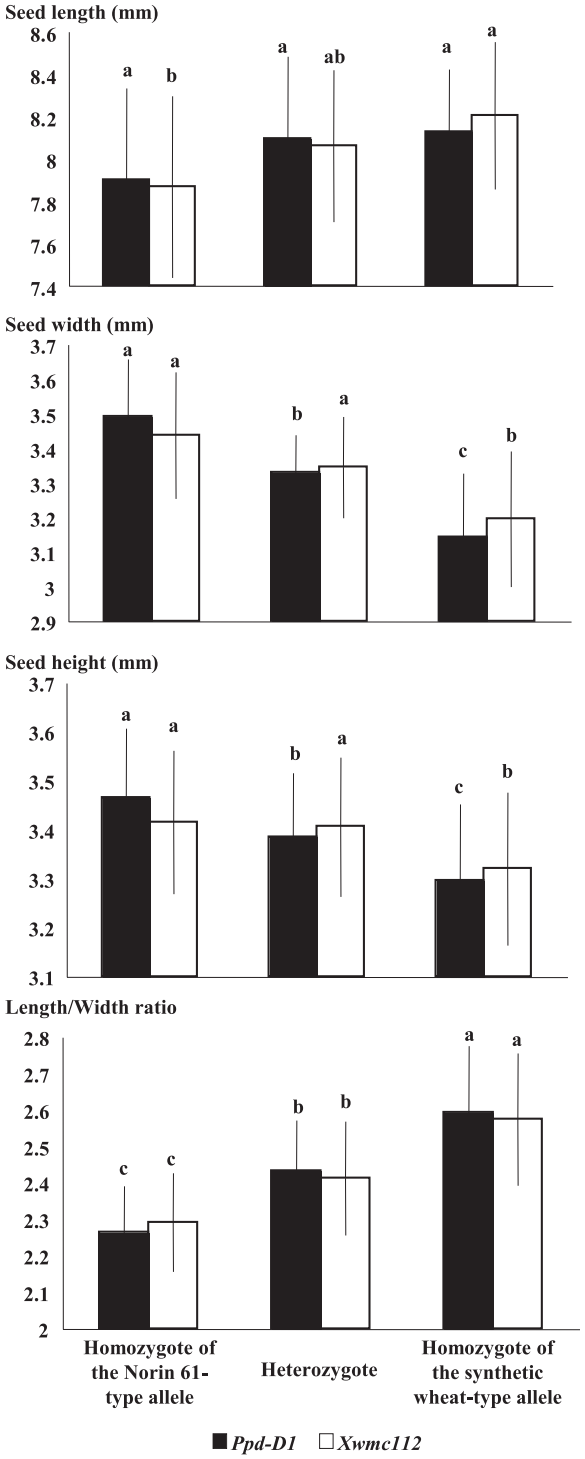

Fig. 4. Effects of three genotypes of *Ppd-D1* (filled bars) and *wmc112* (white bars) at the 2DS QTL on the four traits for wheat grain shape. Means ± standard deviation with the same letter were not significantly different ($P > 0.05$) (Tukey-Kramer HSD test).

notypic variation in each grain and spikelet shape-related trait. Similarly, two QTLs for seed width and three other QTLs for spike length, spikelet density and grain weight were assigned to the 5D QTL region associated with the grain and spikelet shape PC1 (Fig. 3).

Effect of the 2D QTL on wheat grain To study effects of the 2D QTL on grain shape, data of the four traits for grain shape was grouped based on the genotypes at the *Xwmc112* and *Ppd-D1* loci of each F_2 individual. All four traits were significantly ($P < 0.05$) distinct among genotypes at the *Xwmc112* and *Ppd-D1* loci (Fig. 4). The F_2 individuals carrying the Norin 61 homozygous allele at the *Xwmc112* and *Ppd-D1* loci produced significantly rounder grains than those with the synthetic wheat homozygous and heterozygous alleles. The F_2 individuals with the synthetic wheat homozygous allele at the *Xwmc112* and *Ppd-D1* loci showed significantly slenderer grains than other individuals. These results demonstrated that the 2D QTL in the *Xwmc112* and *Ppd-D1*

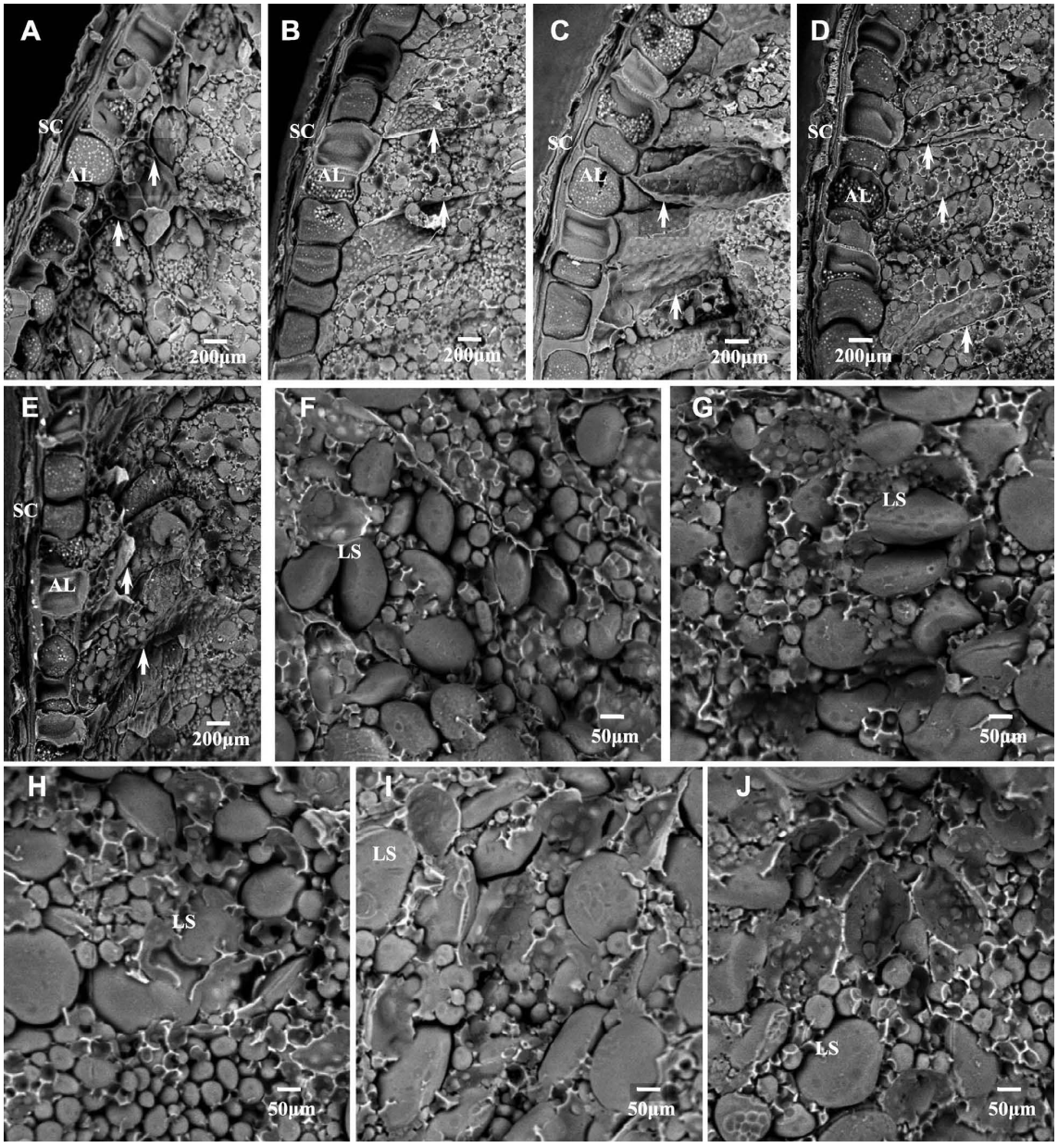

Fig. 5. Scanning electron microscopic observation of transverse sections of wheat grains. Photos of the outside (A to E) and inside (F to J) regions are represented. Arrowheads indicate cell wall. SC; seed coat, AL; aleurone layer, LS; large starch granule. (A, F) Norin 61. (B, G) Synthetic wheat line (Ldn x PI476874). (C, H) F_2 #42, containing the Norin 61-type allele of *Ppd-D1*. (D, I) F_2 #85, containing the synthetic wheat-type allele of *Ppd-D1*. (E, J) F_2 #83, containing the synthetic wheat-type *Ppd-D1* allele and the Norin 61-type *Xwmc112* allele.

chromosomal region significantly affects the difference in grain shape between Norin 61 and the synthetic wheat.

To compare filling of starch granules in grains between Norin 61 and the synthetic wheat, transverse sections of wheat grains were observed using a scanning electron microscope. The synthetic wheat (Ldn x PI476874) exhibited a thinner seed coat than Norin 61, and cells were more ordered under the aleurone layer of the synthetic wheat than in Norin 61 (Fig. 5). However, the thickness of the seed coat and the cell arrangement under the aleurone layer were unrelated to the genotype at the *Xwmc112* and *Ppd-D1* loci. Large and small starch granules filled the inside of the endosperm in grains of Norin 61 and the synthetic wheat, and no allelic differences in the filling of starch granules were observed to be significant in the F_2 individuals.

DISCUSSION

Improvement of grain shape after common wheat speciation Grain shape and size are included in the main components of the domestication syndrome in cereals. A long, thin primitive grain was transformed into a wider, shorter modern grain during wheat domestication (Gegas et al., 2010). Our observations supported previous results; grain shape was longer and thinner in wild tetraploid wheat, whereas it was shorter and wider in cultivated tetraploid wheat (Table 2, Fig. 1). In addition, grain height was greater, and the length-to-width ratio was smaller in cultivated tetraploid wheat, implying that grain shape became rounder during wheat domestication. Common wheat exists only as its cultivated form, meaning as an innate cultivar (Matsuoka, 2011). In fact, the common wheat grains were significantly shorter and rounder than the cultivated tetraploid wheat grains (Table 2, Fig. 1). On the other hand, the synthetic hexaploid wheat provided grains similar to those of cultivated tetraploid wheat, although the D genome of *Ae. tauschii* was artificially added to the AB genomes of tetraploid wheat through allohexaploidization. If the synthetic hexaploids represent the primitive form of common wheat, it could be assumed that grain shape of modern common wheat was improved from the tetraploid-type morphology to the common wheat-type one during wheat breeding.

Natural variation in grain shape of *Ae. tauschii* was reflected in the hexaploid background of synthetic wheat (Fig. 2). This observation implied that the D genome at least partly affects the grain shape of hexaploid wheat, but the effect of the D genome seemed to be limited because the correlation coefficient values were low, especially in seed length, seed width and length-to-width ratio. Therefore, the tetraploid wheat genome mainly contributed to grain shape in synthetic hexaploid wheat. It has been considered that the A genome predominantly controls morphological traits including spike shape, grain shape, and thickness of empty glumes in allopolyploid wheat (Peng et al., 2003; Zhang et al., 2011). The predominant effects of the A genome on wheat morphology might result in limitation of the D-genome contribution to wheat grain shape.

We identified six QTLs for the first three PC values of grain and spikelet shape on chromosomes 1A, 5A, 1D, 2D and 7D. Of them, the 2D QTL for PC2, located on the 2DS chromosomal region around the *Ppd-D1* locus, mainly contributed to the difference in grain shape between common wheat and the synthetic hexaploid (Table 5, Fig. 3). Thus, the 2DS QTL for PC2 could be strongly related to the morphological improvement of grains from primitive to modern common wheat. The *q* locus on the long arm of chromosome 5A is a major domestication-related gene pleiotropically affecting several morphological traits including glume shape, spike length and plant height (Muramatsu, 1986; Kato et al., 2003). The 5A QTL for PC1 was located at a similar position to *q*, but the phenotypic effect of the 5A QTL was lower than expected from the *Q* effect. Because both mapping parents contained the dominant *Q* allele, the 5A QTL for PC1 is not necessarily identical to the *q* locus. A significant QTL for seed length was reported on chromosome 5B in a population between W7984 (a synthetic hexaploid) and Opata 85 (Breseghello and Sorrelles, 2007), and molecular markers on chromosomes 2D, 5A and 5B were associated with kernel morphology using 95 soft winter wheat cultivars (Breseghello and Sorrelles, 2006). Similarly, many QTLs for grain shape have been found in many previous studies using mapping populations among common wheat cultivars (Dholakia et al., 2003; Sun et al., 2009; Gegas et al., 2010). Based on the chromosomal location of previously identified QTLs for grain shape, the QTLs for PC1 and PC2 on chromosomes 2D and 7D seem to be newly identified.

The 7D QTL for PC1 of grain and spikelet shape coexisted with a QTL for spikelet density (Fig. 3). Barley spike density is under the control of several major genes, and one of them, *dense spike-ar* (*dsp.ar*), is assigned to chromosome 7H (Shahinnia et al., 2012). The *dsp.ar* locus affects not only spike density but also grain size, and a mutant allele of *dsp.ar* leads to compact spikes and smaller grains (Shahinnia et al., 2012). The chromosomal position of our identified QTL for PC1 on chromosome 7D might be orthologous to the barley *dsp.ar* locus.

Contribution of the QTL on chromosome 2D to grain shape improvement The 2DS QTL for PC2 of grain and spikelet shape was positioned near *Ppd-D1*. The chromosomal region of the 2DS QTL significantly contributed to determination of wheat grain shape but not grain size (Fig. 4). Norin 61 contained a photoperiod-insensitive allele in the *Ppd-D1* locus, whereas the

synthetic wheat line (Ldn x PI476874) is sensitive to photoperiod for flowering. Thus, the synthetic wheat showed much later heading and flowering time than Norin 61, and a remarkable QTL with a > 10 LOD score was detected at *Ppd-D1* in the F_2 mapping population (data not shown). The grain-filling process progressed under higher temperatures in the late-flowering synthetic wheat than in early-flowering Norin 61 because the temperature gradually increased in the field in April and May (data not shown). Therefore, it could be assumed that the allelic difference at *Ppd-D1* affected grain filling in the F_2 individuals. Our scanning electron microscope observations of transverse sections of wheat grains indicated that filling of starch granules in the endosperm was independent of the *Ppd-D1* alleles (Fig. 5). No significant difference in internal grain structure was observed, and thus the distinct flowering time could be unrelated to differences in grain shape and size between the *Ppd-D1* alleles. Therefore, *Ppd-D1* can be excluded from candidates for the 2DS QTL for PC2.

Two loci controlling the tenacious glume phenotype, *Tg-B1* (= *Tg2*) and *Tg-D1* (= *Tg1*), have been identified in tetraploid and common wheat (Kerber and Rowland, 1974; Simonetti et al., 1999). *Tg-D1* is located just distally from *Ppd-D1* on 2DS (Jantasuriyarat et al., 2004; Sood et al., 2009; Dvorak et al., 2012). Glumes tenaciously enclose grains in the synthetic hexaploid wheat lines, whereas modern cultivars including Norin 61 are free-threshing wheat. *Tg* is epistatic to *Q*, and the *QQTgTg* genotype has a non-free-threshing phenotype (Matsuoka, 2011). Because the genotype of Ldn, a free-threshing cultivar, is predicted to be *QQtg2tg2*, the synthetic hexaploid wheat lines and Norin 61 are considered to be *QQtg2tg2Tg1Tg1* and *QQtg2tg2tg1tg1*, respectively. The chromosomal position of the 2DS QTL for PC2 of the grain and spikelet shape corresponded to that of *Tg-D1* (Fig. 3). This result strongly suggests that *Tg-D1* controls the differences in spikelet and grain shape between Norin 61 and synthetic wheat, in addition to their threshability, and pleiotropically affects not only glume toughness but also spikelet and grain shape in hexaploid wheat. The toughness of empty glume and lemma probably is related to their morphology and indirectly regulates kernel growth. The tenacious glume of hulled wheat may strongly restrict grain expansion, and soft glumes may enable wheat grain enlargement even beyond the size of the lemma. At the chromosomal region of the 2DS QTL for PC2, QTLs for spike length, grain number and spikelet number co-localized. Significant correlations between glume tenacity and spike shape-related traits have been previously reported (Jantasuriyarat et al., 2004). Thus, the *Tg-D1* locus might have a significant influence on spike shape in hexaploid wheat.

Because *Ae. tauschii* is hulled, primitive hexaploid wheat was hulled, and free-threshing common wheat is considered to have been derived from a hulled ancestor (Matsuoka, 2011; Dvorak et al., 2012). It has been strongly predicted that the tetraploid parent of common wheat was not hulled emmer but a free-threshing form of tetraploid wheat (Dvorak et al., 2012). In addition, hexaploid wheat with the genotype *QQtg2tg2Tg1Tg1* was a transient form that existed for a short period prior to emergence of free-threshing common wheat (Matsuoka, 2011). Therefore, the genetic change from *Tg1Tg1* to *tg1tg1* occurred at an early phase after hexaploid wheat speciation, meaning that wheat grains were rapidly improved to the smaller, rounder phenotype that accompanied the formation of free-threshing wheat. This idea is based on the assumption that the 2DS QTL for PC2 of spikelet and grain shape is identical to *Tg-D1*. To test the assumption, molecular cloning of *Tg-D1* and production of a near-isogenic line of the 2DS QTL using tightly linked markers would be required.

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REFERENCES

Araki, E., Ikeda, T. M., Ashida, K., Takata, K., Yanaka, M., and Iida, S. (2009) Effects of rice flour properties on specific loaf volume of one-loaf bread made from rice flour with wheat vital gluten. Food Sci. Technol. Res. **15**, 439–448.

Asakura, N., Mori, N., Nakamura, C., and Ohtsuka, I. (2009) Genotyping of the *Q* locus in wheat by a simple PCR-RFLP method. Genes Genet. Syst. **84**, 233–237.

Beales, J., Turner, A., Griffiths, S., Snape, J. W., and Laurie, D. A. (2007) A *Pseudo-Response Regulator* is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). Theor. Appl. Genet. **115**, 721–733.

Breseghello, F., and Sorrells, M. E. (2006) Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics **172**, 1165–1177.

Breseghello, F., and Sorrells, M. E. (2007) QTL analysis of kernel size and shape in two hexaploid mapping populations. Field Crop Res. **101**, 172–179.

Campbell, K. G., Bergman, C. J., Gualberto, D. G., Anderson, J. A., Giroux, M. J., Hareland, G., Fulcher, R. G., Sorrells, M. E., and Finney, P. L. (1999) Quantitative trait loci associated with kernel traits in a soft x hard wheat cross. Crop Sci. **39**, 1184–1195.

Dholakia, B. B., Ammiraju, J. S. S., Singh, H., Lagu, M. D., Röder, M. S., Rao, V. S., Dhaliwal, H. S., Ranjekar, P. K., Gupta, V. S., and Weber, W. E. (2003) Molecular marker

analysis of kernel size and shape in bread wheat. Plant Breed. **122**, 392–395.

Dvorak, J., Deal, K. R., Luo, M. C., You, F. M., von Borstel, K., and Dehghani, H. (2012) The origin of spelt and free-threshing hexaploid wheat. J. Heredity **103**, 426–441.

Evers, A. D., Cox, R. I., Shaheedullah, M. Z., and Withey, R. P. (1990) Predicting milling extraction rate by image analysis of wheat grains. Asp. Appl. Biol. **25**, 417–426.

Fuller, D. Q. (2007) Contrasting patterns in crop domestication and domestication rates: Recent archaeobotanical insights from the Old World. Ann. Bot. **100**, 903–924.

Gegas, V. C., Nazari, A., Griffiths, S., Simmonds, J., Fish, L., Orford, S., Sayers, L., Doonan, J. H., and Snape, J. W. (2010) A genetic frame work for grain size and shape variation in wheat. Plant Cell **22**, 1046–1056.

Hori, K., Kobayashi, T., Shimizu, A., Sato, K., Takeda, K., and Kawasaki, S. (2003) Efficient construction of high-density linkage map and its application to QTL analysis in barley. Theor. Appl. Genet. **107**, 806–813.

Huang, X., Qian, Q., Liu, Z., Sun, H., He, S., Luo, D., Xia, G., Chu, C., Li, J., and Fu, X. (2009) Natural variation at the *DEP1* locus enhances grain yield in rice. Nat. Genet. **41**, 494–497.

Jantasuriyarat, C., Vales, M. I., Watson, C. J. W., and Riera-Lizarazu, O. (2004) Identification and mapping of genetic loci affecting the free-threshing habit and spike compactness in wheat (*Triticum aestivum* L.). Theor. Appl. Genet. **108**, 261–273.

Kajimura, T., Murai, K., and Takumi, S. (2011) Distinct genetic regulation of flowering time and grain-filling period based on empirical study of D-genome diversity in synthetic hexaploid wheat lines. Breed Sci. **61**, 130–141.

Kato, K., Sonokawa, R., Miura, H., and Sawada, S. (2003) Dwarfing effect associated with the threshability gene *Q* on wheat chromosome 5A. Plant Breed. **122**, 489–492.

Kerber, E. R., and Rowland, G. G. (1974) Origin of free threshing character in hexaploid wheat. Can. J. Genet. Cytol. **16**, 145–154.

Klingenberg, C. P. (2010) Evolution and development of shape: integrating quantitative approaches. Nat. Rev. Genet. **11**, 623–635.

Kobayashi, F., Takumi, S., and Handa, H. (2010a) Identification of quantitative trait loci for ABA responsiveness at the seedling stage associated with ABA-regulated gene expression in common wheat. Theor. Appl. Genet. **121**, 629–641.

Kobayashi, H., Yanaka, M., and Ikeda, T. M. (2010b) Exogenous methyl jasmonate alters trichome density on leaf surfaces of Rhodes grass (*Chloris gayana* Kunth). J. Plant Growth Regul. **29**, 506–511.

Kosambi, D. D. (1944) The estimation of map distance from recombination values. Ann. Eugen. **12**, 172–175.

Kovach, M. J., Sweeney, M. T., and McCouch, S. R. (2007) New insights into the history of rice domestication. Trends Genet. **23**, 578–587.

Lander, E. S., Green, P., and Abrahamson, J. (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1**, 174–181.

Langlade, N. B., Feng, X., Dransfield, T., Copsey, L., Hanna, A. I., Thébaud, C., Bangham, A., Hudson, A., and Coen, E. (2005) Evolution through genetically controlled allometry space. Proc. Natl. Acad. Sci. USA **102**, 10221–10226.

Liu, Q., Ni, Z., Peng, H., Song, W., Liu, Z., and Sun, Q. (2007) Molecular mapping of a dominant non-glaucousness gene from synthetic hexalploid wheat (*Triticum aestivum* L.). Euphytica **155**, 71–78.

Matsuoka, Y. (2011) Evolution of polyploid *Triticum* wheats under cultivation: the role of domestication, natural hybridization and allopolyploid speciation in their diversification. Plant Cell Physiol. **52**, 750–764.

Matsuoka, Y., and Nasuda, S. (2004) Durum wheat as a candidate for the unknown female progenitor of bread wheat: an empirical study with a highly fertile F_1 hybrid with *Aegilops tauschii* Coss. Theor. Appl. Genet. **109**, 1710–1717.

Matsuoka, Y., Takumi, S., and Kawahara, T. (2007) Natural variation for fertile triploid F_1 hybrid formation in allohexaploid wheat speciation. Theor. Appl. Genet. **115**, 509–518.

Matsuoka, Y., Nishioka, E., Kawahara, T., and Takumi, S. (2009) Genealogical analysis of subspecies divergence and spikelet-shape diversification in central Eurasian wild wheat *Aegilops tauschii* Coss. Plant Syst. Evol. **279**, 233–244.

Mizuno, N., Hosogi, N., Park, P., and Takumi, S. (2010) Hypersensitive response-like reaction is associated with hybrid necrosis in interspecific crosses between tetraploid wheat and *Aegilops tauschii* Coss. PLoS ONE **5**, e11326.

Muramatsu, M. (1986) The *vulgare* super gene, *Q*: its universality in *durum* wheat and its phenotypic effects in tetraploid and hexaploid wheats. Can. J. Genet. Cytol. **28**, 30–41.

Peng, J., Ronin, Y., Fahima, T., Röder, M. S., Li, Y., Nevo, E., and Korol, A. (2003) Domestication quantitative trait loci in *Triticum dicoccoides*, the progenitor of wheat. Proc. Natl. Acad. Sci. USA **100**, 2489–2494.

Salamini, F., Ozkan, H., Brandolini, A., Schafer-Pregl, R., and Martin, W. (2002) Genetics and geography of wild cereal domestication in the near east. Nat. Rev. Genet. **3**, 429–441.

Shahinnia, F., Druka, A., Franckowiak, J., Morgante, M., Waugh, R., and Stein, N. (2012) High resolution mapping of Dense spike-ar (*dsp.ar*) to the genetic centromere of barley chromosome 7H. Theor. Appl. Genet. **124**, 373–384.

Shomura, A., Izawa, T., Ebana, K., Ebitani, T., Kanegae, H., Konishi, S., and Yano, M. (2008) Deletion in a gene associated with grain size increased yields during rice domestication. Nat. Genet. **40**, 1023–1028.

Simonetti, M. C., Bellomo, M. P., Laghetti, G., Perrino, P., Simeone, R., and Blanco, A. (1999) Quantitative trait loci influencing free-threshing habit in tetraploid wheats. Genet. Resour. Crop Evol. **46**, 267–271.

Somers, D. J., Isaac, P., and Edwards, K. (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. **109**, 1105–1114.

Song, X. J., Huang, W., Shi, M., Zhu, M. Z., and Lin, H. X. (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat. Genet. **39**, 623–630.

Sood, S., Kuraparthy, V., Bai, G., and Gill, B. S. (2009) The major threshability genes soft glume (*sog*) and tenacious glume (*Tg*), of diploid and polyploid wheat, trace their origin to independent mutations at non-orthologous loci. Theor. Appl. Genet. **119**, 341–351.

Sun, X. Y., Wu, K., Zhao, Y., Kong, F. M., Han, G. Z., Jiang, H. M., Huang, X. J., Li, R. J., Wang, H. G., and Li, S. S. (2009) QTL analysis of kernel shape and weight using recombinant inbred lines in wheat. Euphytica **165**, 615–624.

Takumi, S., Naka, Y., Morihiro, H., and Matsuoka, Y. (2009a) Expression of morphological and flowering time variation through allopolyploidization: an empirical study with 27 wheat synthetics and their parental *Aegilops tauschii*

accessions. Plant Breed. **128**, 585–590.

Takumi, S., Nishioka, E., Morihiro, H., Kawahara, T., and Matsuoka, Y. (2009b) Natural variation of morphological traits in wild wheat progenitor *Aegilops tauschii* Coss. Breed Sci. **59**, 579–588.

Torada, A., Koike, M., Mochida, K., and Ogihara, Y. (2006) SSR-based linkage map with new markers using an intraspecific population of common wheat. Theor. Appl. Genet. **112**, 1042–1051.

Tsunewaki, K., and Ebana, K. (1999) Production of near-isogenic lines of common wheat for glaucousness and genetic basis of this trait clarified by their use. Genes Genet. Syst. **74**, 33–41.

Wang, S., Basten, C. J., and Zeng, Z. B. (2007) Windows QTL cartographer 2.5. Department of Statics, North Carolina State University, Raleigh, NC. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm

Zhang, Z., Belcram, H., Gornicki, P., Charles, M., Just, J., Huneau, C., Magdelenat, G., Couloux, A., Samain, S., Gill, B. S., Rasmussen, J. B., Barbe, V., Faris, J. D., and Chalhoub, B. (2011) Duplication and partitioning in evolution and function of homoeologous *Q* loci governing domestication characters in polyploid wheat. Proc. Natl. Acad. Sci. USA **108**, 18737–18742.

Zhou, Y., Zhu, J., Li, Z., Yi, C., Liu, J., Zhang, H., Tang, S., Gu, M., and Liang, G. (2009) Deletion in a quantitative trait gene *qPE9-1* associated with panicle erectness improves plant architecture during rice domestication. Genetics **183**, 315–324.