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Research Information

Nucleotide diversity of a *grain softness protein 1* gene *GSP-U1* in wild wheat relative *Aegilops umbellulata*

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Grain hardness is important for determination of wheat grain quality, and the grain hardness variation is mainly controlled by a *Hardness* (*Ha*) locus on the short arm of chromosome 5D in common wheat (Sourdille et al. 1996). The *Ha* locus contains three genes; two puroindoline protein-encoding genes *Pina-D1* and *Pinb-D1*, and *grain softness protein 1* (*GSP-1*) (Rahman et al. 1994; Giroux and Morris 1998). Out of the three genes, the allelic differences of *Pina-D1* and *Pinb-D1* are associated with cultivar differences of the soft and hard types of common wheat grains (Giroux and Morris 1998; Morris 2002; Ikeda et al. 2005). Tetraploid wheat including durum wheat shows a very hard kernel texture because of lacking puroindolines (Gautier et al. 2000). The *GSP-1* genes are present on chromosomes 5A and 5B and expressed in tetraploid wheat (Bhave and Morris 2008), and found in various *Aegilops* and *Triticum* species (Chen et al. 2005; Morris et al. 2013). The *GSP-1*-encoding protein is structurally related to puroindolines after post-translational proteolysis, and does not interact with polar lipid in contrast to Pina and Pinb (Elmorjani et al. 2013). However, the role of *GSP-1* in grain hardness is still under discussion.

Aegilops umbellulata Zhuk., a diploid wild wheat relative with UU genome, has been used as a genetic resource for wheat breeding (Friebe et al. 1996). Many alleles of *Pina* and *Pinb*, were found in *Ae. umbellulata* (Cuesta et al. 2015; Okada et al. 2018), and mature grains of *Ae. umbellulata* showed a hard texture due to nonsynonymous substitutions in the *Pin* alleles or lack of PIN protein accumulation (Okada et al. 2018).

Table 1. Intraspecific distribution of the *GSP-1* alleles in 58 *Ae. umbellulata* accessions

GSP-1 types (allele names)	Accessions of *Ae. umbellulata*
GSP-U1b (*GSP-U1-II*)	KU-2752, KU-2770, KU-4001, KU-4028, KU-4042, KU-4052, KU-4068, KU-4075, KU-4080, KU-4086, KU-5901, KU-5910, KU-5911, KU-5913, KU-5924, KU-5928, KU-5931, KU-12200, KU-12202, KU-12701, KU-11482a, KU-8-7
GSP-U1d (*GSP-U1-IV*)	KU-4006, KU-4007, KU-4010, KU-4017, KU-4026, KU-4039, KU-4043, KU-4074, KU-4079, KU-4103, KU-8-5
GSP-U1e (*GSP-U1-V*)	KU-2932, KU-4024, KU-4035, KU04046, KU-4070, KU-4081, KU-4087, KU-4088, KU-4019, KU-5947, KU-5948, KU-5949, KU-12204
GSP-U1f (*GSP-U1-VI*)	KU-4030
GSP-U1g (*GSP-U1-VII*)	KU-2760, KU-12198
GSP-U1h (*GSP-U1-VIII*)	KU-4041
GSP-U1i (*GSP-U1-IX*)	KU-5954
GSP-U1j (*GSP-U1-X*)	KU-4025
GSP-U1k (*GSP-U1-XI*)	KU-12180, KU-12186, KU-12189
GSP-U1l (*GSP-U1-XII*)	KU-12190, KU12207a
GSP-U1l (*GSP-U1-XII*)	KU-12190, KU12207a

KU: Plant Germ-Plasm Institute, Faculty of Agriculture, Kyoto University, Japan.

Up to now, four *GSP-U1* alleles have been identified in *Ae. umbellulata* (Cuesta et al. 2015). However, relation of the *GSP-U1* allelic difference to grain hardness still remains to be unknown. Here, we analyzed the *GSP-U1* nucleotide sequences in various *Ae. umbellulata* accessions, and compared the *GSP-U1* alleles with grain hardness data that we reported previously (Okada et al. 2018).

Table 2. Summary of nucleotide variation at the *GSP-1* locus in *Ae. umbellulata*

	Entire region	Synonymous	Nonsynonymous
# of sites	504	110.514	384.486
# of variable sites	14	4	10
π	0.00519	0.00436	0.00549
θ	0.00606	0.00782	0.00562
Tajima's *D*	-0.42 ^{NS}	-0.97 ^{NS}	-0.065 ^{NS}
Fu and Li's *D**	-0.97 ^{NS}	-1.26 ^{NS}	-0.51 ^{NS}

NS: not significant

Total DNA was extracted from leaves of 58 *Ae. umbellulata* accessions (Table 1). Seeds of the *Ae. umbellulata* accessions were supplied by the National BioResource Project-Wheat (Japan, www.nbrp.jp). The *GSP-U1* region was amplified with the specific PCR primer set, 5'– TCGAGGAAAATTGCAGATTCAGTG–3' and 5'– CCATGGTCACATTTATTGGTCACA–3', as referred by Cuesta et al. (2015). The *GSP-U1* region was amplified by ExTaq polymerase (Takara Bio, Shiga, Japan) in each *Ae. umbellulata* accession, and the PCR product was sequenced with an applied biosystem 3730xl DNA analyzer (Applied Biosystems, Foster City, CA). Multiple sequences alignment and population genetic analyses were performed using DnaSP v5 (Librado and Rozas 2009) software according to our previous study (Okada et al. 2017). The nucleotide sequences of the *GSP-U1* region were deposited in the DDBJ database (accession numbers LC424195 - LC424202).

The *GSP-U1* nucleotide sequences were determined in the 58 accessions, and compared with that of *GSP-D1a* from a common wheat cultivar 'Falcon' (Rahman et al. 1994). In total, 14 polymorphic sites were observed. Out of them, ten sites caused nonsynonymous substitution (Table 1). Nucleotide diversity (π) of *GSP-U1* was 0.00519, which was similar to that of *Pina-U1* and slightly lower than *Pinb-U1* (Okada et al. 2018). The tests of Tajima's *D* (Tajima 1989) and Fu and Li's *D** (Fu and Li 1993) were conducted to test neutrality at *GSP-1* genes. The test of Tajima's D gave no significant result for *GSP-1* (Table 2), suggesting that the *GSP-U1* nucleotide variation is neutral in the *Ae. umbellulata* population. In the previous study, four alleles were respectively reported for *GSP-U1* in *Ae. umbellulata* (Cuesta et al. 2015). In this study, novel eight *GSP-U1* alleles were found, and *GSP-U1* sequences were grouped into 12 types including four known alleles [*GSP-U1a* (*GSP-U1-I*), *GSP-U1b* (*GSP-U1-II*), *GSP-U1c* (*GSP-U1-III*) and *GSP-U1d* (*GSP-U1-IV*)] (Fig. 1, Table 1). The two previously reported alleles, *GSP-U1a* and *GSP-U1b*, were not found in the 58 *Ae. umbellulata* accessions examined in this study. All of the *GSP-U1* sequences were inconsistent with that of the *GSP-1* functional allele *GSP-D1a*.

An amino acid change from glycine to serine at position 46 in Pinb results in alteration of the wheat grain texture (Giroux and Morris 1997, 1998). GSP-1 contains a hypothetical AAI domain at positions from 63 to 164, which might play important roles in lipid transfer and storage in seeds (Charvolin et al. 1999; Samuel et al. 2002; Rico et al. 1996). A recent report showed no interaction of GSP-1 from the B-genome copy of common wheat with lipid (Elmorjani et al. 2013). In the AAI domain of GSP-U1, two serine residues were changed to proline or leucine at position 107 and to asparagine at position 142 of GSP-U1 (Fig. 1). The amino acid substitution at position 107 was observed among all of the *GSP-U1* alleles. In addition, an amino acid change from threonine to serine at position 85 was found in the *GSP-U1a* and *GSP-U1c* alleles. Therefore, the GSP-U1 function such as lipid-binding property could be different from GSP-D1a. Although function of GSP-1 in wheat endosperm still remains to be unknown, much more *GSP-1* alleles could be discovered by further sequence analyses using other *Aegilops* species.

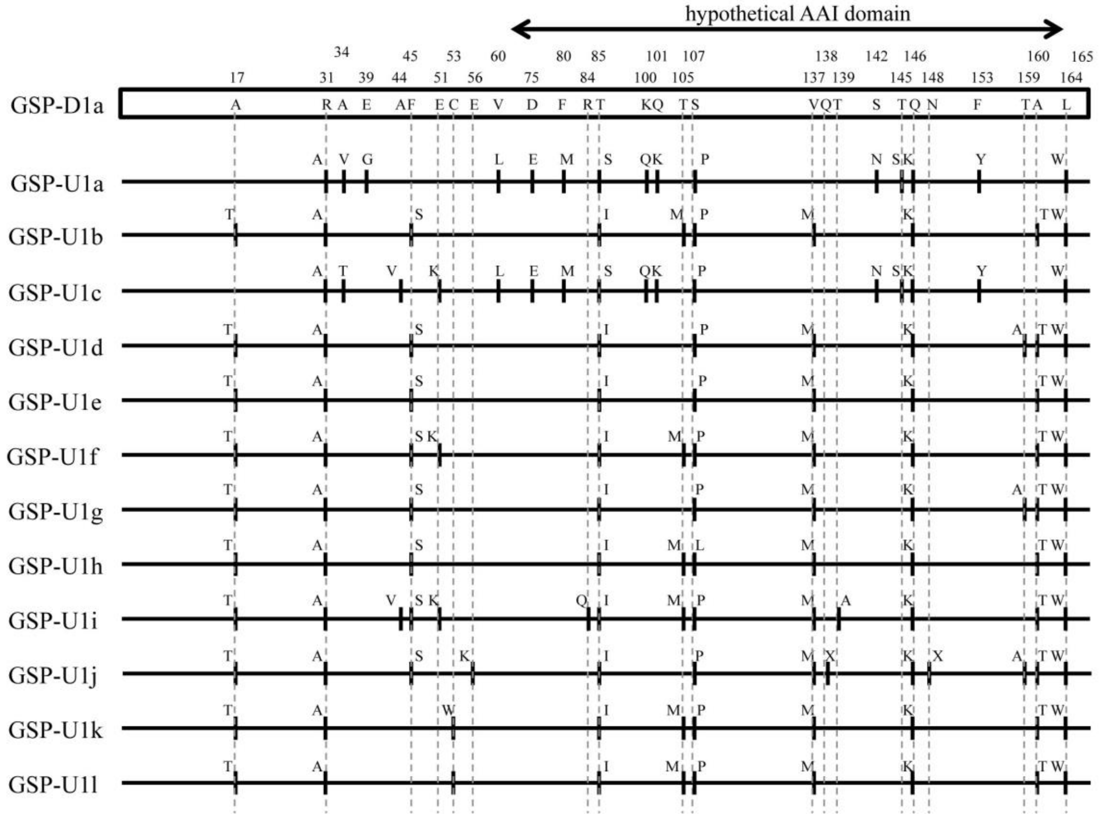

Figure 1. Allelic diversity of the *GSP-1* gene in *Ae. umbellulata*. Primary structures of putative GSP-1 are shown. The structure of GSP-1 encoded by GSP-D1a of *Ae. tauschii* is designated as rectangles at the top of the figure. The structures of GSP-1 encoded by *GSP-U1* alleles are shown as horizontal lines. The vertical bars with one-letter abbreviations of amino acids correspond to the sites with amino acid substitutions between GSP-1 encoded by *GSP-D1a* and *GSP-U1* alleles.

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