Wheat Inf. Serv. 101, 2006. www.shigen.nig.ac.jp/ewis

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Electronic Newsletter for Wheat Researchers

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Research Information

Phosphorylation of wheat chloroplast-targeting COR/LEA proteins via 50-kDa protein kinase

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Abstract

Cold acclimation, an adaptive process for developing low temperature/freezing tolerance, is an important agronomic trait that is regulated by the concerted expression of a series of *Cor* (cold responsive)/*Lea* (late embryogenesis abundant) genes. To study biochemical characteristics of paralogous wheat COR/LEA proteins, WCOR14 and WCOR15, their mode of phosphorylation was examined by *in vitro* and in-gel assays. The assays showed that these COR/LEA proteins were phosphorylated by 50-kDa wheat protein kinase(s), the process of which likely contributes to their chloroplast targeting.

Cold acclimation is an adaptive process for developing low temperature (LT)/freezing tolerance in over-wintering plants. The process involves a number of biochemical and physiological changes (Levitt 1980; Guy et al. 1985), which are regulated by LT through changes in gene expression. Among a number of LT-responsive genes, the *Cor* (cold-responsive)/*Lea* (late-embryogenesis-abundant) family is the most well characterized gene family that comprises major LT-signaling components and can contribute to the significant development of freezing tolerance (Thomashow 1999). Two wheat *Cor*/*Lea* genes, *Wcor14* and *Wcor15*, encode chloroplast-targeting proteins and are associated with cold acclimation (Tsvetanov et al. 2001; Takumi et al. 2003; Shimamura et al. 2005). *Wcor15* shows a high level of homology with *Wcs19*, whose transcript level is positively correlated with the relative reduction state of photosystem II in wheat (Gray et al. 1997). WCOR14, an ortholog of barley BCOR14b (Crosatti et al. 1999), contains the same signal peptide for chloroplast targeting at the N-terminal as WCOR15.

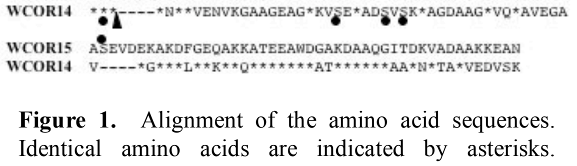

Figure 1. Alignment of the amino acid sequences. Identical amino acids are indicated by asterisks. Boxes with a double and a single line show putative chloroplast signal peptides and a 14-3-3 protein recognition motif, respectively. The site of an intron insertion is indicated by a filled triangle. Filled circles represent putative phosphorylation sites.

NDong et al. (2002) reported that the highly homologous signal peptides of WCOR14 and WCS19 contained one putative 14-3-3 protein recognition motif. This amino acid motif was conserved in WCOR15 (Fig. 1). In this motif, an S-residue was predicted as a phosphorylation site by the NetPhos version 2.0 software (http://genome.cbs.dtu.dk/services/NetPhos). Besides this, there were four and two other putative phosphorylation sites in WCOR14 and WCOR15, respectively (Fig. 1).

To study whether WCOR14 and WCOR15 are the phosphorylation targets, *in vitro* phosphorylation assay was performed. A winter wheat cultivar 'Mironovskaya 808' (abbreviated as M808) was grown in a controlled-climate cabinet at 25 ˚C with a 16 h photoperiod at a light intensity of 110-120 µm photons $m^{-2} s^{-1}$ provided by cool white fluorescence lamps (the standard condition). Conditions for cold acclimation were according to Ohno et al. (2001). Total proteins (10 µg) from cold-acclimated and non-acclimated M808 seedlings were mixed with 10 µg of the recombinant WCOR14 and WCOR15 proteins that were synthesized in *E. coli* and purified by affinity chromatography according to Kobayashi et al. (2004). The phosphorylation reaction was performed for 2 h at 25 ˚C in solution containing 50 mM HEPES-KOH (pH 7.5), 10 mM $MgCl_2$, 2 mM $MnCl_2$ and 10 µCi [γ-^{32}P] ATP. An aliquot (10 µL) of each reaction was analyzed by SDS-PAGE and visualized by autoradiography. This *in vitro* phosphorylation assay clearly showed that both WCOR proteins were phosphorylated (Fig. 2A). The level of phosphorylation was much higher in WCOR14 than in WCOR15, which seemed to reflect the number of putative phosphorylation sites in these WCOR proteins. A weak signal was observed at the position of ca. 50-kDa, suggesting that this 50-kDa protein(s) is the putative kinase with multifunctional and autophosphorylation activity.

To confirm that the 50-kDa protein(s) represents the wheat kinase(s) associated with the phosphorylation of WCOR14 and WCOR15, in-gel assay was conducted. Total proteins (20 µg) from cold-acclimated and non-acclimated M808 seedlings were fractionated on a SDS-PAGE gel containing 0.1 mg mL^{-1} of WCOR14 or WCOR15 proteins. Renaturable in-gel protein kinase activity (Usami et al. 1995) was assayed by incubating the gel for 1 h in buffer containing 40 mM Tris-HCl (pH 8.0), 50 mM NaCl, 20 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 2mM DTT, 50 mM ATP, and 10 µCi [γ-^{32}P] ATP. Phosphorylated proteins were detected by autoradiography. The assay showed that the two WCOR proteins in fact were phosphorylated by kinase with 50-kDa in size. LT treatment however did not affect the level of phosphorylation of these COR proteins (Fig. 2B), which agreed with the result of *in vitro* assay (Fig. 2A). Under the LT condition, this kinase was active irrespective of the light conditions, which seemed to be in contrast with the LT-inducible and light-stimulated expression of these COR proteins (Shimamura et al. 2005). Under the non-acclimated condition, however, the light illumination enhanced the level of phosphoryaltion of both WCOR proteins. The regulation of this kinase activity by LT and light has to be further clarified.

Generally, the 14-3-3 recognition motifs are phosphorylated and continuously interact with the 14-3-3 proteins (May and Soll 2000). The binding of the 14-3-3 proteins to the signal peptides is necessary for the chloroplast precursor proteins to be efficiently transported into chloroplasts (May and Soll 2000). Since both of the WCOR14 and WCOR15 proteins contain a putative 14-3-3 recognition motif in the chloroplast-targeting signal and WCOR15 at least is targeted into chloroplasts in transgenic tobacco plants (Takumi et al. 2003), these phosphorylated proteins might interact with the 14-3-3 proteins to be efficiently transported into chloroplasts of both monocotyledonous and dicotyledonous plants.

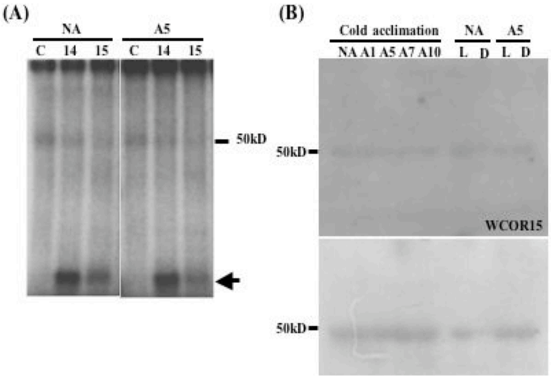

Figure 2. Phosphorylation of WCOR15 and WCOR14. (A) *in vitro* phosphorylation assay. C, negative control; NA, non-acclimated; A5, 5-d cold-acclimated; 14, WCOR14; 15, WCOR15. (B) In-gel phosphorylation assay. Protein extracts from M808 seedling leaves were separated on SDS-PAGE gels containing 0.1 mg/ml of recombinant WCOR15 or WCOR14. NA, non-acclimated; A1 to A10, 1-d to 10-d cold-acclimated; L, 16h light/8h dark; D, continuous dark.

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Wheat Inf. Serv. 101: 4-7, 2006. www.shigen.nig.ac.jp/ewis

Research Information

ABA sensitivity in seedlings of two novel mutants with reduced dormancy of a common wheat cultivar 'Norin 61'

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Abstract

Abscisic acid (ABA) plays important roles in mediating stress responses and in acquiring dormancy of seeds. To study ABA sensitivity in two non-dormant mutants of common wheat, seedling growth in ABA-containing water, ABA-responsibility of *Cor* (cold-responsive)/*Lea* (late-embryogenesis-abundant) gene expression and freezing tolerance after cold acclimation were analyzed in the mutant lines and their parental cultivar 'Norin 61' (N61). In spite of their non-dormant phenotype, no significant difference was observed in post-germination growth of the parental and the mutant lines. Our results indicated that the two mutations of ABA sensitivity mainly affect developing seeds.

Abscisic acid (ABA) regulates important aspects of plant growth and development including environmental stress tolerance and seed maturation and dormancy (Leung and Giraudat 1998; Finkelstein et al. 2002). Regulatory mechanisms of ABA-dependent gene expression have been studied using a *vp1* (*viviparous1*) mutant of maize and *abi* (*ABA-insensitive*) mutants of *Arabidopsis thaliana*, both of which showed reduced levels of seed dormancy and sensitivity to exogenous ABA for inhibition of germination (Leung and Giraudat 1998). ABA biosynthesis is required for seed maturation and dormancy during seed development, while in vegetative tissues ABA is synthesized *de novo* mainly in response to drought and high salinity stresses (Xiong and Zhu 2003; Shinozaki et al. 2003). Many genes as components of stress signaling pathways are induced by exogenous ABA in *Arabidopsis* and rice (Xiong et al. 2002; Rabbani et al. 2003). LT and ABA regulatory pathways are not completely independent. Several *Cor*/*Lea* genes are in fact responsive to exogenous ABA, and their promoter sequences commonly contain ABRE (ABA responsive element) (Lång and Palva 1992; Shinozaki and Yamaguchi-Shinozaki 2000).

However, information on roles of ABA in the regulation of ABRE-containing genes under LT conditions is still limited in wheat and its related species. An ABA-insensitive, non-dormant line of common wheat, EH47-1, was derived from an ABA sensitive and dormant line, 'Kitakei-1354' (Kitakei), a single dominant mutant by EMS (ethylmethan sulfonate) mutagenesis (Kawakami et al. 1997). Embryos of the mutant line loose sensitivity to ABA during the later half process of seed maturation, while embryos of the parental line maintain the sensitivity even after maturity. Comparative studies of freezing tolerance after cold acclimation and *Cor*/*Lea* gene expression between Kitakei and EH47-1 suggested that ABA sensitivity contributes to determine the basal level of freezing tolerance in wheat (Kobayashi et al. in press). To obtain more information on the relationship between ABA and cold/freezing tolerance, two novel wheat mutant lines of reduced seed dormancy were identified and analyzed in this study.

Two mutant lines (RSD16-1 and RSD32) of common wheat (*Triticum aestivum* L.) were selected through NaN_3-induced mutagenesis of a strong dormant cultivar 'Norin 61' (N61) based on the increased germination rate at DAP40 (40 days-after-pollination) (Fig. 1A). N61 seeds at DAP40 showed a very low germination rate thus strong dormancy, whereas both

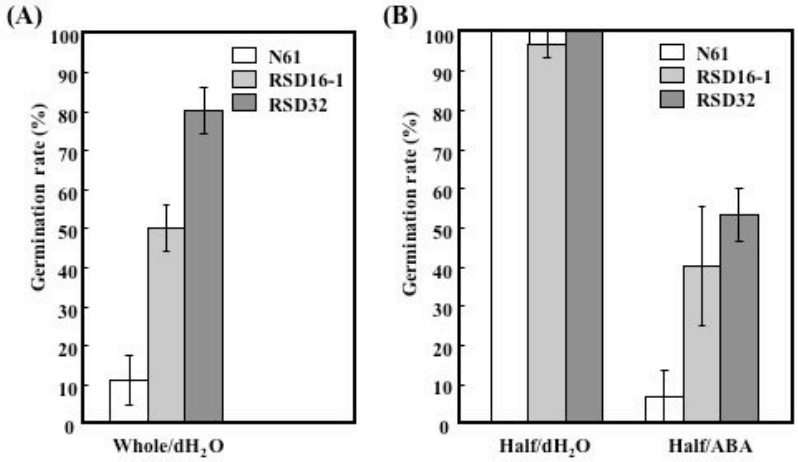

Figure 1. Characterization of the two mutant lines, RSD16-1 and RSD32, derived from NaN$_3$-treated N61. (A) Comparison of germination rates without exogenous supply of ABA of whole grains at 40 DAP in N61 and the mutant lines. (B) Comparison of germination rates with or without ABA (100 μM) of half grains containing embryos at 40 DAP in N61 and the mutant lines. Fourteen seeds per line were assayed, and the whole experiments were repeated three times. The small bars represent standard errors.

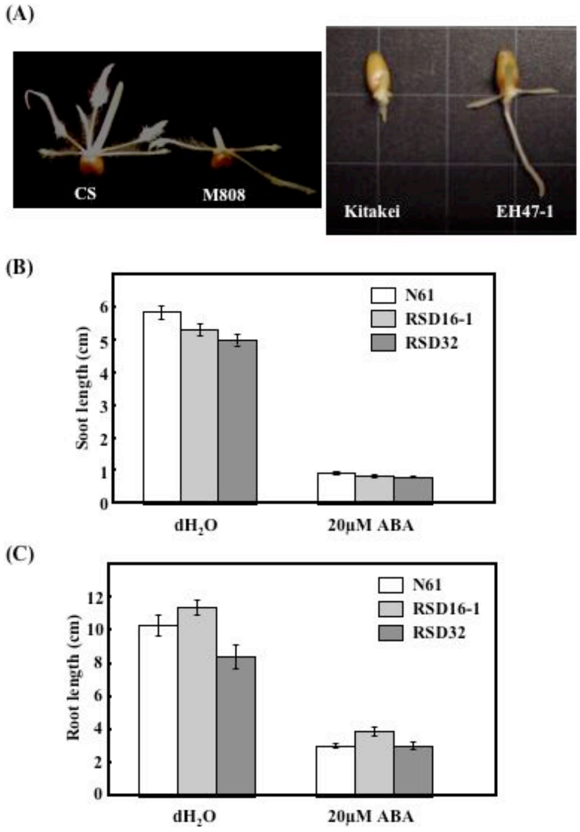

Figure 2. Bioassay for ABA sensitivity during post-germination growth. (A) Comparison of CS and M808 (left), and Kitakei and EH47-1 (right) under an ABA-treated condition. Comparisons of shoot length (B) and primary root length (C) between N61 and the mutant lines (RSD16-1 and RSD32). Ten seeds per line were germinated with and without 20 μM ABA at 20˚C for 6 days. The whole experiments were repeated four times. The small bars represent standard errors.

mutant lines showed dramatically reduced dormancy at this stage. Half seeds with embryos germinated normally without exogenous supply of ABA and thus completely lost dormancy in both N61 and the mutants (Fig. 1B), indicating that ABA was supplied from the endosperm. Exogenous ABA (100μM) reduced the germination rate of the half seeds of N61. Both mutant lines showed lower levels of sensitivity to the inhibitory effect of exogenous ABA than N61.

In bioassay for ABA sensitivity based on post-germination growth, 10 seeds from each line were placed in plastic petri dish with distilled water or 20 μM ABA solution and incubated at 20˚C in the darkness. On the sixth day, lengths of shoots and primary roots were recorded. In our previous study, this bioassay could efficiently monitor differences in ABA sensitivity among wheat accessions based on seedling growth (Kobayashi et al. in press; Fig. 2A). According to this parameter, exogenous ABA greatly reduced shoot and root lengths in both N61 and the mutants, but no significant differences were observed between them (Fig. 2B, C). This result indicated that ABA sensitivity estimated based on the germination rate of developing seeds was not necessarily corresponding to that based on the post-germination growth.

ABA sensitivity in wheat seedlings was next studied based on the level of ABA-induced gene expression. For this, ABA treatment was performed by spraying 7-day-old seedlings of N61 and the mutants grown under standard conditions (Ohno et al. 2001) with a solution of 20 μM ABA containing 0.1% (w/v) Tween 20. Total RNA was extracted 2 hour after the ABA treatment. Steady state levels of transcripts of two wheat *Cor*/*Lea* genes, *Wdhn13* and *Wrab17*, were studied by northern blot analysis using the corresponding cDNA clones as ^{32}P-labelled probes. These cDNA clones were previously characterized (Tsuda et al. 2000; Ohno et al. 2003; Kobayashi et al. 2004). The northern blots showed that *Wdhn13* and *Wrab17* were clearly induced by exogenous ABA in the mutant lines as well as in Norin 61 (Fig. 3). Bases on the amount of transcripts, *Wdhn13* was more sensitive to ABA than *Wrab17*, but there were no significant differences between N61 and the mutant lines.

These results indicate that the two mutations of ABA sensitivity mainly affect developing seeds similar to *Arabidopsis abi3* mutation (Finkelstein et al. 2002). *Arabidopsis ABI3*, which is an ortholog of a maize *VP1*, has pleiotropic effects on seed maturation, regulation of sensitivity to ABA inhibition of germination, expression of some seed-specific genes, acquisition of desiccation tolerance, and dormancy (Giraudat et al. 1992; Parcy et al. 1994). Transcripts of wheat *Vp-1* genes are alternatively spliced (Mckibbin et al. 2002). Further expression study of the wheat *Vp-1* genes should be required to clarify the relationship between the three mutations and the *Vp-1* loci.

For evaluation of freezing tolerance, 7-day-old seedlings of N61 and the mutants were subjected to bioassay according to Kobayashi et al. (2004). No significant difference however was found in cold acclimated seedlings at 4°C for 21 days between N61 and the mutants. Association of ABA sensitivity with cold/freezing tolerance therefore remained unclear. Isolation of additional mutations affecting ABA sensitivity should be needed to study roles of ABA in abiotic stress responses in wheat.

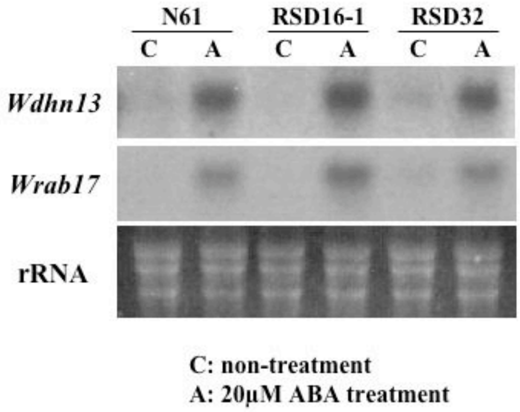

Figure 3. Comparison of the *Wdhn13* and *Wrab17* transcripts accumulation between N61 and its non-dormant mutants (RSD16-1 and RSD32). Seedlings were treated with 20 μM ABA, and total RNA was isolated two hour after the treatment. Northern blots were probed with ^{32}P-labelled cDNAs of the *Wdhn13* and *Wrab17* genes. rRNAs are used as a control.

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Wheat Inf. Serv. 101: 8-10, 2006. www.shigen.nig.ac.jp/ewis

Research Information

Coleoptile color variation in *Aegilops tauschii*. I. Afghanistan, Iran, and Pakistan

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I report coleoptile color variation observed among *Aegilops tauschii* accessions collected in Afghanistan, Iran, and Pakistan. In the December of 2003 and 2004, one seed was sown for each accession in a pot in a slightly-heated greenhouse at Fukui Prefectural University. Coleoptile color was checked by eye each year and classified as red (i.e., anthocyanin pigmentation) or green (i.e., no anthocyanin pigmentation) (Table 1).

In the 74 accessions tested, there are four accessions (KU2050, KU2051, KU2058, and KU2113) that have green coleoptiles. Of those, KU2050, KU2051, and KU2058 are from the Maimana region in Afghanistan. This result is consistent with Kihara et al's observation for green coleoptiles to be found in seven out of 15 populations of the Maimana region (Kihara et al. 1965).

The *Ae. tauschii* (syn. *Ae. squarrosa* L.) accessions used in this study are maintained at the Plant Germ-plasm Institute of Kyoto University. I thank Taihachi Kawahara for the materials.

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Table 1. Coleoptile color of *Ae. tauschii* accessions collected in Afghanistan, Iran, and Pakistan

Accession No.	Country	Locality	Coleoptile color
KU-20-6	Pakistan	8 km SW of Quetta	Red
KU-20-7	Iran	2 km N of Karaj (Suburbs of Tehran)	Red
KU-20-10	Iran	9 km NW of Ramsar (Chalus – Rasht)	Red
KU-2001	Pakistan	Suburbs of Quetta	Red
KU-2003	Pakistan	Suburbs of Quetta	Red
KU-2006	Pakistan	Quetta – Chaman	Red
KU-2008	Pakistan	Chaman	Red
KU-2010	Afghanistan	Kandahar – Jaldak	Red
KU-2012	Afghanistan	Jaldak	Red
KU-2016	Afghanistan	Jaldak – Ghazni	Red
KU-2018	Afghanistan	31 km NE of Ghazni (Ghazni – Kabul)	Red
KU-2022	Afghanistan	4 km E of Kabul (Kabul – Jallabad)	Red
KU-2025	Afghanistan	22 km N of Doski (Kabul – Pulikhumri)	Red
KU-2027	Afghanistan	16 km NW of Pulikhumri (Pulikhumri – Haibak)	Red
KU-2028	Afghanistan	26 km NW of Pulikhumri (Pulikhumri – Haibak)	Red
KU-2032	Afghanistan	38 km NW of Pulikhumri (Pulikhumri – Haibak)	Red

Table 1. *(continued)*

KU-2035	Afghanistan	42 km NW of Pulikhumri (Pulikhumri - Haibak)	Red
KU-2039	Afghanistan	47 km NW of Pulikhumri (Pulikhumri - Haibak)	Red
KU-2042	Afghanistan	54 km NW of Pulikhumri (Pulikhumri - Haibak)	Red
KU-2043	Afghanistan	36 km S of Andkhui (Andkhui - Maimana)	Red
KU-2044	Afghanistan	13 km N of Maimana (Andkhui - Maimana)	Red
KU-2050	Afghanistan	Suburbs of Maimana	Green
KU-2051	Afghanistan	16 km W of Maimana (Maimana - Qala Naw)	Green
KU-2056	Afghanistan	20 km to 35 km W of Maimana (Maimana - Qala Naw)	Red
KU-2058	Afghanistan	40 km W of Maimana (Maimana - Qala Naw)	Green
KU-2059	Afghanistan	75 km W of Maimana (Maimana - Qala Naw)	Red
KU-2061	Afghanistan	118 km SW of Maimana (Maimana - Qala Naw)	Red
KU-2063	Afghanistan	151 km SW of Maimana (Maimana - Qala Naw)	Red
KU-2066	Afghanistan	30 km NE of Qala Naw (Maimana - Qala Naw)	Red
KU-2068	Iran	Suburbs of Ghazvin, Tehran - Ghazvin	Red
KU-2069	Iran	2 km N of Karaj (Suburbs of Tehran)	Red
KU-2074	Iran	5 km W of Behshahr (Sari - Behshahr)	Red
KU-2075	Iran	15 km E of Behshahr (Behshahr - Gorgan)	Red
KU-2076	Iran	8 km W of Gorgan	Red
KU-2077	Iran	Darkalah, near Aliabad (Gorgan - Khoshyailagh)	Red
KU-2078	Iran	E of Aliabad (Gorgan - Khoshyailagh)	Red
KU-2079	Iran	E of Aliabad (Gorgan - Khoshyailagh)	Red
KU-20-8	Iran	25 km WSW of Firuzkuh	Red
KU-2080	Iran	Gharaghaj near Shah-passand (Gorgan - Khoshyailagh)	Red
KU-2082	Iran	NE of Koshyailagh (Gorgan - Khoshyailagh)	Red
KU-2083	Iran	Koshyailagh	Red
KU-2086	Iran	11 km WSW of Firuzkuh (Gorgan - Khoshyailagh)	Red
KU-2087	Iran	15 km NE of Sari (Sari - Behshahr)	Red
KU-2088	Iran	36 km NE of Sari (Sari - Behshahr)	Red
KU-20-9	Iran	5 km W of Behshahr (Sari - Behshahr)	Red
KU-2090	Iran	Behshahr	Red
KU-2091	Iran	3 km N of Babulsar (Babulsar - Sari)	Red
KU-2092	Iran	3 km N of Babulsar (Babulsar - Sari)	Red
KU-2093	Iran	51 km W of Babulsar (Babulsar - Chalus)	Red
KU-2096	Iran	51 km W of Babulsar (Babulsar - Chalus)	Red
KU-2097	Iran	51 km W of Babulsar (Babulsar - Chalus)	Red
KU-2098	Iran	Ramsar (Chalus - Rasht)	Red
KU-2100	Iran	29 km NW of Ramsar (Chalus - Rasht)	Red
KU-2101	Iran	43 km NW of Ramsar (Chalus - Rasht)	Red
KU-2102	Iran	52 km NW of Ramsar (Chalus - Rasht)	Red
KU-2103	Iran	13 km SSE of Rasht (Chalus - Rasht)	Red
KU-2104	Iran	Pahlavi	Red
KU-2105	Iran	12 km NW of Pahlavi (Pahlavi - Astara)	Red
KU-2106	Iran	14 km NW of Pahlavi (Pahlavi - Astara)	Red
KU-2107	Iran	12 km N of Hashtpar (Pahlavi - Astara)	Red
KU-2108	Iran	12 km N of Hashtpar (Pahlavi - Astara)	Red
KU-2109	Iran	Astara (Pahlavi - Astara)	Red
KU-2110	Iran	32 km SW of Astara (Astara - Ardabil)	Red
KU-2111	Iran	Ardabil	Red

Table 1. *(continued)*

KU-2112	Iran	19 km SW of Ardabil (Ardabil - Surab)	Red
KU-2113	Iran	Suburbs of Mahabad	Green
KU-2115	Iran	50 km NNW of Mahabad (Mahabad - Rezaiyeh)	Red
KU-2116	Iran	32 km S of Khoy (Rezaiyeh - Khoy)	Red
KU-2118	Iran	Khoy	Red
KU-2120	Iran	12 km N of Marand (Khoy - Tabriz)	Red
KU-2121	Iran	42 km NW of Tabriz (Khoy - Tabriz)	Red
KU-2122	Iran	Mixed in chicken feed, Tabriz	Red
KU-2124	Iran	Techalousse (near Chalus)	Red
KU-2126	Iran	Techalousse (near Chalus)	Red

Wheat Inf. Serv. 101: 11-12, 2006. www.shigen.nig.ac.jp/ewis

Research Information

Multiple origins of U genome in two UM genome tetraploid *Aegilops* species, *Ae. biuncialis* and *Ae. ovata*

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The genera *Aegilops* and *Triticum* include a lot of polyploid species that contain various combinations of genomes. Recent molecular analyses have gradually revealed that some of the polyploid species was not formed by a single polyploidization event, but by several times events. In other words, some polyploids have polyphyletic multiple origins within a species. Table 1 summarizes the molecular evidence for multiple origins of polyploid species in the genera *Aegilops* and *Triticum* reported to date. As you see in Table 1, multiple origins are likely to be the general rule for polyploid species in these genera.

Here, we report a new evidence for the multiple origins of the U genome in two UM genome tetraploid species, *Ae. biuncialis* and *Ae. ovata*, based on the PCR-RFLP of the U genome-specific U31 fragment that we developed previously (Kadosumi et al. 2005). We investigated the PCR-RFLP of the U31 fragment of 48 accessions each of *Ae. biuncialis* and *Ae. ovata*. All the accessions used were the collection of the Plant Germ-plasm Institute, Graduate School of Agriculture, Kyoto University. The allelic variation of U31 fragment observed in the two species was shown in Table 2. We found common alleles between the U genome diploid *Ae. umbellulata* and *Ae. biuncialis*, and between *Ae. umbellulata* and *Ae. ovata*. These results suggested the multiple origins of the U genome in the two tetraploid species.

Sequencing analysis revealed that the allele II detected in *Ae. biuncialis* has the CCAG at the *Msp*I site, which was also found in 21 accessions of *Ae. umbellulata*. This result strongly supports the hypothesis that the allele II of *Ae. biuncialis* has been derived from *Ae. umbellulata* by secondary polyploidization, although the order of the polyploidization is indeterminable. The multiple origins of the M genome in *Ae. biuncialis* has been reported by Chee et al. (1995), but our present study is the first

Table 1. Previous reports on multiple origins of polyploid species in the genera *Aegilops* and *Triticum*

Species	Genome	Polyphyletic genome	Reference
Ae. cylindrica	CCDD	D genome	Caldwell et al. (2004)
Ae. triuncialis	CCUU	Chloroplast U genome U genome, Chloroplast	Murai and Tsunewaki (1986) Chee et al. (1995) Vanichanon et al. (2003)
Ae. biuncialis	UUMM	M genome	Chee et al. (1995)
Ae. columnaris	UUMM	U genome	Kadosumi et al. (2005)
Ae. triaristata	UUMM	U genome	Kadosumi et al. (2005)
T. aestivum	AABBDD	D genome	Talbert et al. (1998)

Table 2. Number of accessions for each allele of U31 in the U genome diploid *Ae. umbellulata* and two UM genome tetraploids *Ae. biuncialis* and *Ae. ovata*

U31 Allele[a]	*Ae. umbellulata*[b]	*Ae. biuncialis*	*Ae. ovata*
allele I	35 (0.49)[c]	46 (0.96)	5 (0.10)
allele II	27 (0.38)	2 (0.04)	0 (0.00)
allele III	8 (0.11)	0 (0.00)	0 (0.00)
allele IV (null)	2 (0.03)	0 (0.00)	43 (0.90)
Total	72 (1.00)	48 (1.00)	48 (1.00)

[a]: Classification of U31 alleles is following the description of Kadosumi et al. (2005); allele I = normal size product with an *Msp*I site, allele II = normal size product without an *Msp*I site, allele III = shorter size product without *Msp*I site, and allele IV = not amplified.

[b]: The values for *Ae. umbellulata* is cited from Kadosumi et al. (2005).

[c]: Frequency of each allele in the species is shown in the parenthesis.

report of multiple origins of the U genome in this species. As for *Ae. ovata*, the alleles shared with *Ae. umbellulata* were alleles I and IV. Since the allele IV is null allele, we could not determine the sequence of this allele. Therefore, strictly speaking, it is not conclusive that the allele IV in *Ae. ovata* has been derived from *Ae. umbellulata*. To ascertain the multiple origins of *Ae. ovata*, more information on other loci are necessary.

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Wheat Inf. Serv. 101: 13-14, 2006. www.shigen.nig.ac.jp/ewis

Research Information

Chromosome breakage in wheat plants doubly hemizygous for the gametocidal genes derived from *Aegilops speltoides* and *Ae. sharonensis*

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Gametocidal (Gc) genes, introduced to common wheat from *Aegilops* species, cause chromosome breakage (for review, see Endo 1990). Cytological observations revealed that chromosome breakage is a post-meiotic event in plants hemizygous for a Gc gene (Finch et al. 1984; Nasuda et al. 1999). Tsujimoto (1995) summarized available data and described interaction between different Gc genes. Regarding to the interaction between Gc genes on homoeologous group-2 and -4 chromosomes, he concluded that Gc genes on group-4 chromosomes are epistatic to those on group-2 chromosomes. Moreover, he pointed out that chromosome breakage was enhanced in plants doubly hemizygous for Gc genes on different homoeologous groups. These conclusions were drawn from cytological observations of structurally aberrant chromosomes in the progeny.

Here, I describe chromosome breakage occurred in a doubly hemizygous wheat plants for Gc genes from chromosomes 2S of *Ae. speltoides* and $4S^{sh}$ of *Ae. sharonensis*. These Gc genes were translocated to the tips of long arms of homoeologous wheat chromosomes, being described as T2B-2S and T4B-$4S^{sh}$ by Nasuda et al. (1999). Cytological observation of male gametogenesis of a double-monosomic substitution line (dMT2B-2S/4B-$4S^{sh}$) revealed that there are three kinds of pollen grain with different degrees of chromosome breakage; 'normal (none)', 'severe', and 'moderate' (Figure 1). In normal pollen grains (Figure 1, top), no chromosome fragments and/or bridges were observed in anaphase of first postmeiotic mitosis. The pollen grains belonged to 'severe' chromosome breakage had many chromosome fragments between spindle poles (Figure 1, middle) and the pollen grains categorized to 'moderate' chromosome breakage had only two or three chromosome fragments (Figure 1, bottom). In total, I observed 110 pollen grains. The numbers of pollen grains in each category were; 33 'normal', 55 'moderate', and 25 'severe'. These numbers well fit to the segregation ration of 1:2:1 (χ^2=1.491, df=2), indicating that 'severe' chromosome breakage occurred in gametophytes lacking both Gc chromosomes.

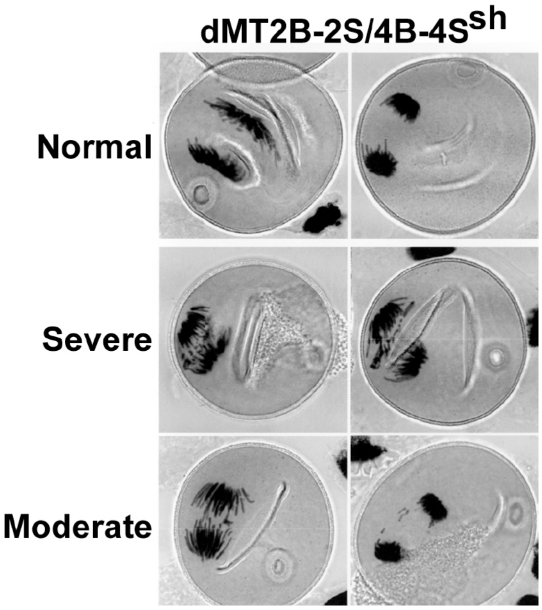

Figure 1. Pollen grains at anaphase of first pollen mitosis in doubly hemizygous wheat plants for Gc genes. Three kinds of pollen grains were observed; those with no (top), severe (middle), and moderate (bottom) chromosome breakage (top). The methodology of cytological observation was as written by Nasuda et al. (1999).

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Wheat Inf. Serv. 101: 15-20, 2006. www.shigen.nig.ac.jp/ewis

Meeting Reports

The Triticeae Meeting of Japan

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The Wheat Genetics Symposium of Japan (WGSJ) has been restarted as The Triticeae Meeting of Japan (TMJ). A joint meeting between WGSJ and The Molecular Biology Meeting of Triticeae was held in Tottori University, 2004. In that meeting, it made a decision that two meetings should be united and held annually in several places in Japan. The new meeting covers wide range of fields such as molecular biology, genomics, molecular cytogenetics, physiology, breeding and genecology and evolution.

The first joint meeting named “The Triticeae Meeting of Japan” was held on November 11 and 12, 2005 in Kyoto Prefectural Institute of Agricultural Biotechnology, Seika-cho Kyoto. 94 researchers including students participated to the TMJ meeting (Fig. 1). We had three special lectures, seven oral presentations and 28 poster presentations. The meeting was active and impressive, mainly because many young scientists participated to the meeting and made valuable discussions.

The abstracts and titles are presented below. I hope that many scientists are going to attend to the next meeting and give nice data, even if those are not complete.

Thank you again, indeed.

Figure 1. The first Triticeae Meeting of Japan - Participant Group Photo.

ABSTRACTS & TITLES

Oral Presentation

O1. Regulation of plastid gene transcription in higher plants

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The plastid genome is transcribed by nuclear-encoded (NEP) and plastid-encoded (PEP) RNA polymerases. Interestingly, PEP is responsible for transcription of a subset of plastid genes, including photosystem I and II genes, whereas many housekeeping genes are transcribed by both PEP and NEP. Analogous to the eubacterial enzyme, PEP is a multi-subunit enzyme composed of a catalytic core complex ($\alpha 2\beta\beta'\beta''$) and a sigma factor that confers transcription specificity. *Arabidopsis* contains six nuclear-encoded sigma factors (AtSIG1 – AtSIG6), although the core subunits are encode by plastid genome. Recent molecular studies have shown that each plastid sigma factor likely has a specific function. Like eubacterial sigma factors, plastid sigma factors can also be grouped into general factors responsible for transcription of standard PEP-dependent genes and specialized factors involved in the recognition of unique PEP promoters. AtSIG6 likely acts as a general sigma factor and supports early chloroplast development in young cotyledons. By contrast, AtSIG5 is structurally distinct among plastid sigma factors and is probably involved in transcription of photosystem reaction center genes, including *psbD*, *psbA* and *psaAB*. Furthermore, it has been demonstrated that AtSIG2 is responsible for transcription of several tRNA genes. It is presumed that nuclear-encoded multiple sigma factors may play a crucial role in transcriptional regulation of plastid-encoded photosystem and tRNA genes in higher plants.

O2. Current status and future prospect of chloroplast transformation in higher plants

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Chloroplast transformation is an attractive technology for obtaining the plants with new useful characteristics for human being. Transplastomic plants generally have a number of advantages over conventional nuclear transformants, e.g. 1) strict containment of the transgene due to maternal inheritance of chloroplasts and to the lack of gene flow through pollen, 2) ability to accumulate a large amount of transgene's product in the chloroplast, 3) multigene engineering through the appropriate use of an "operon", 4) absence of gene silencing and position effects on the transgene, and so on.

In our laboratory, being supported by the Foundation for Bio-venture Research Center from the Ministry of Education, Culture, Sports, Science and Technology, Japan, the attempts to obtain useful recombinant plants by chloroplast transformation technology have been done, and several transplastomic tobacco plants have already been produced. Our transplastomic plants belong to either categories A) the plants with improved characteristics, or B) the plants producing pharmaceutical products, or C) the plants which can be used for phytoremediation process. In this meeting, transplantomic tobacco with *Arabidopsis* fatty acids desaturase gene (*fad8*) was presented as an example of the plant in the category A. PCR and Southern analyses showed that *fad8* gene is integrated into the chloroplast genome as expected, and preliminary data on fatty acid composition indicated that the ratio of triunsaturated-/diunsaturated-fatty acids was slightly increased in the transplastomic tobacco. Another example of the category A is transplastomic tobacco plants with soybean's ferritin gene. PCR and Southern analyses confirmed that the gene is integrated into the chloroplast genome, and quantitative measurement of iron contents showed that the amounts of iron are increased three times in the transplastomic plants compared to the wild-type tobacco. A single successful example in the category B is a transplastomic tobacco with *hrd* gene encoding thrombin inhibitor hirudin. PCR, Southern, Northern and Western analyses showed the *hrd* gene is integrated into the chloroplast genome correctly, and mRNA and protein are accumulated in the chloroplast, although biological activity of chloroplast hirudin have yet to be elucidated. Our laboratory presently is involved with the construction of transplastomic plants in the category C. Radish genes involving phytochelatin synthesis, such as glutathione synthetase (*gs*), γ-glutamylcysteine synthetase (*gsh1*) and phytochelatin synthase (*pcs*), are in our hand, and they will be delivered to the tobacco chloroplast genome, to test the possibility of obtaining a hyperaccumlator for heavy metals.

O3. Various functions of aquaporins in MIP gene family

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Aquaporins are membrane proteins that facilitate water movement across bio-membranes. Aquaporins are suggested to mediate not only water but also other molecules transports. Aquaporin genes are known as Major Intrinsic Protein (MIP) genes.

In barley EST database, putative 24 MIPs (contigs) were identified and 11 genes of plasma membrane type aquaporin (PIPs) were detected by PCR. Expression of these 11 PIPs were investigated under salt (NaCl), osmotic (manitol), heavy metals ($CuCl_2$ and $CdCl_2$), and oxidative (H_2O_2) stresses. One of them, HvPIP2;1, was most abundant and its protein expression was also analyzed. It was confirmed that *HvPIP2;1*, encoded water channel activity in *X. laevis* oocytes injected with *HvPIP2;1* cRNA. Transcripts and proteins of *HvPIP2;1* were reduced in barely roots under salt stress. Over-expression of *HvPIP2;1* increased the shoot/root ratio and raised salt sensitivity in transgenic rice plants, indicating *HvPIP2;1* is involved in the cellular mechanism of salt tolerance. Over-expression of the *HvPIP2;1* also increased internal CO_2 conductance and CO_2 assimilation in the leaves of transgenic rice plants, suggesting that *HvPIP2;1* permeates CO_2 in addition to H_2O. Recent reports from other researchers suggested that aquaporins were involved in the flood-induced reduction of root water uptake, chilling-induced decrease of root water permeability, and other many physiological functions in plants.

O4. Why is wheat so unique? - Understanding a mechanism of gluten formation based on a comparison of seed storage proteins among cereals.

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Wheat seed proteins consist of glutenin and gliadin, which belongs to prolamin superfamily. The prolamin superfamily also includes rice prolamins, rye secalins, maize zeins and barley hordeins. Among these prolamin proteins, only wheat prolamins can form a gluten macropolymer. Based on a sequence comparison, it was clear that glutenin, gliadin, hordein and secalin but rice prolamins contained glutamine-rich tandem repetitive sequences. This structure might be involved in protein elasticity. The number of cysteine residues in these proteins involved in inter- and intra- disulfide bonds was varied even within a same species. Fractionation of seed proteins by aqueous alcohol with DTT (insoluble polymeric proteins) or without DTT (soluble polymeric and monomeric proteins) showed that barley hordeins did not form insoluble polymers, probably due to a very low amount of D-hordein, which shared a similar structure to wheat high-molecular-weight glutenin subunits (HMW-GSs). Since HMW-GSs is known to be important to form a structural framework of gluten polymers, the amount of D-hordein might be critical for hordein polymerization.

O5. Wheat production and target of wheat breeding program in Japan

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Japan consume about 5.7million ton of wheat a year. Domestic wheat production is 860 thousand ton. Improvement of Japanese wheat quality has been demanded by food industry. Wheat breeders are trying the improvement of quality and resistance to disease or severe environment. Pre-harvest sprouting (PHS) causes severe damage to wheat quality. Zenkouji-komugi is the most resistant to PHS in Japanese wheat cultivars. Our objective is to raise all cultivars to the resistance. Fusarium head bright (FHB) resistance is the most important breeding target. The disease causes significant economic losses, especially production of toxin. We are trying to introduce the resistant genes from Sumai 3. However the resistance for FHB may be inadequate for actual wheat cultivation.

The amylose content is associated with elasticity of white salt noodle. The *wx-B1b* is responsible for a good noodle quality. High molecular weight glutenin subunits relate bread making quality. Low molecular weight glutenin are not fully understood the relations of noodle and bread making quality. We are trying to introduce the optimum glutenin subunits compositions for each food products. Grain hardness of Japanese soft wheat cultivars is softer than that of foreign wheat cultivars. The soft grain make small flour particle by milling. Arabinoxylan content of flour is associated with flour yield. We believe that both low arabinxylan content and a large flour particle achieve a good milling property.

O6. Expression profile of gliadin and glutenin gene families in hexaploid wheat by large-scale EST analysis

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For discerning the expression patterns of individual members of storage protein gene families in hexaploid wheat, we analyzed comprehensive ESTs by the bioinformatics method. The genes for α/β-gliadins and low-molecular-weight (LMW)-glutenin subunits (GS) were selected from the EST database. The SNP sites among each member of both genes were traced by the alignment. The combinations of the SNPs allowed us to assign those haplotypes into their homoeologous chromosomes by allele-specific PCR. The phylogenetic analysis showed that both of the genes rapidly diverged after differentiation of three genomes, namely A, B and D. Expression patterns of these genes were estimated based on the frequencies of ESTs. These storage protein genes were expressed only in the seed developing stages. The α/β-gliadin genes revealed at least two distinct expression patterns during the course of seed maturation. While early expressing genes of α/β-gliadin and LMW-GS genes showed similar expression patterns and were preferentially expressed from D genome, late expressing genes of α/β-gliadin were drastically expressed from the A genome. Phylogenetic relationships and their expression patterns were not correlated. These evidences suggest that expressions of the two gene families are independently regulated among multi-gene members, and α/β-gliadin genes should possess novel regulation system(s) in addition to the prolamin box.

O7. Molecular mechanisms of the accumulation process of seed storage proteins in rice and other cereals

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The endosperm tissues of cereal seeds are the principal storage organs for protein, oil, and carbohydrate. In other words, cereal endosperm cells are extremely important cells that provide basic food for humankind. An understanding of the formation mechanisms of endosperm tissues of cereals is extremely important in stabilizing world crop production. The rice endosperm is an ideal tissue for basic studies of cereal endosperm development, because rice has been selected as a model plant for studying the genome science.

In the ripening period, rice seed stored many kinds of substances, i.e. starch, protein, mineral, and oil. These substances are rapidly biosynthesized mainly in the endosperm cells, leading the formation of the cellular organelles as a specific deposition site for each storage substance. The physiological role of storage proteins is to provide nutrients such as nitrogen and sulfur for germination. Up to 95% of the endosperm protein is deposited in protein granules called protein body (PB). Most of the stored rice protein is glutelin, globulin and prolamin, although these proteins are accumulated in different PB. The type-I protein body (PB-I) is spherical and contains prolamin polypeptide. On the other hand, the type-II protein body (PB-II) is rich in glutelin and globulin. These protein bodies develop in an endosperm cell basically at the same time, and different types of storage proteins are correctly sorted into the proper deposition sites. In this study, I consider the molecular mechanisms of seed storage protein synthesis and accumulation in rice compared with other cereals.

O8. Diversity of *Lolium temulentum*, an associated weed of wheat and barley, in Malo, Ethiopia

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Lolium temulentum, darnel, is an associated weed of wheat and barley. In Malo, south-western Ethiopia, where people maintain traditional ways of subsistence by growing various kinds of crops and livestock by conducting sustainable shifting cultivation, man's impacts, especially cereal cultivation, on the diversity of darnel was surveyed. The grains of darnel are either awned or awnless, and the awnless is dominant over the awned. Awned form was found in emmer wheat, and awnless one was generally associated with bread, macaroni and rivet wheat. In Malo, grain cleaning is done by winnowing and subsequent hand removal of contaminants. Emmer wheat has non-free threshing grains, and the other three crops have free threshing grains. Awned darnel's grain morphology is similar to that of emmer wheat grain, and the awnless darnel grain resembles the free threshing grains of bread, macaroni and rivet wheat. Separating awned darnel grains from emmer wheat grains is difficult, as is separating awnless grains from free threshing wheat grains. The free threshing wheat grains contaminated with darnel grains are sown in the emmer wheat field because the boundaries between the two fields are unclear. Crop seed exchange and contamination of crop grains with darnel grains during storage or seeding of crops lead to unintended artificial gene flow of darnel and consequently conserve the genetic diversity of darnel.

O9. Cytogenetic analysis of wheat-barley hybrids

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In wheat-barley intergeneric hybridization, many cytogenetically interesting phenomena are observed. In this presentation, I summarize our work on the production and genetic analysis of wheat-barley hybrids. Genetic variation in crossability with wheat was detected among barley germplasms. QTL analysis detected four genes controlling the crossability of barley with wheat. In a certain cross combination with an enhanced crossability, barley chromosome 4H was preferentially eliminated in hybrid plants, while another cross combination showed preferential elimination of barley chromosome 1H. In wheat-barley hybrids, not all barley dominant genes controlling morphological traits are expressed, indicating that complicated genetic interaction is operating between the genomes of wheat and barley. A barley gene on the long arm of chromosome 1H causes sterility in hybrids with wheat (*Shw*). This gene was mapped both genetically and physically by introducing a series of aberrant barley 1H chromosomes into wheat followed by molecular marker analysis. Finally, homoeologous chromosome pairing between barley chromosome 5H and wheat homoeologues was successfully induced by nullisomy 5B. However, all recombinant chromosomes recovered had a cross-over point in the distal region of the group 5 chromosomes. This may hamper transfer of proximally located barley genes into the wheat genetic background with a minimum amount of barley chromatin.

O10. Chromosomal localization pattern of the heterochromatin protein 1 (HP1) in wheat

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In eukaryote nuclei, genomic DNA builds up the macrostructure with histones and other non-histone proteins, which is called chromatin. The highly condensed region of the chromatin structure is referred as heterochromatin that functionally involves in nuclear organization, chromosomal segregation, and gene regulation. Most of genes in heterochromatic regions are transcriptionally inactive, and this state of chromatin is epigenetically inherited through mitotic and meiotic cell divisions. Heterochromatin Protein 1 (HP1) is one of the non-histone proteins associated with heterochromatins. Recent studies in yeasts revealed that HP1 plays a key role in formation and maintenance of heterochromatin by intermediating heterochromatin-specifically modified histone H3 (methylated at lysine 9) and other heterochromatin components.

In this study, genes encoding for HP1 homologues were isolated from common wheat *Triticum aestivum*, and chromosomal locations and expression patterns of the wheat HP1 genes were determined. The indirect fluorescent antibody technique was applied to determine localization patterns of HP1 protein on mitotic chromosomes in wheat and related species. The results showed that the wheat HP1 homologues are encoded by homoeologous genes locating on the group-7 chromosomes and all the genes are expressed constitutively. Wheat HP1 proteins were localized to centromere in diploids and tetraploids, but were distributed to entire length of the chromosomes in hexaploid wheat. The localization patterns of di- or tri-methylated histone H3K9 and other modified histones (monomethylated H3K27, trimethylated histone H4K20, phosphorylated histone H3S10, phosphorylated H3S28 dimethylated H3K4, and acetylated H3K9) did not coincided with those of HP1 in wheat plants at different ploidy levels. In addition, localization of HP1 to chromatin did not change after treatment with 5'-azacytidine, an inhibitor of DNA methylation although the localization patterns of some modified histones changed. These findings suggest that localization of HP1 might not be regulated directly by methylation status of either histone H3 at Lysin 9 or DNA.

Poster Presentation

P1. Nishio, Z.[1], K. Takata[2], T. Tabiki[1], M. Ito[1], N. Iriki[1], T. Ban[3] (1. NARCH, 2. NARCW, 3. JIRCAS) The effect of marker assisted selection on Fusarium head blight resistance in winter wheat of Hokkaido.

P2. Tonooka,T., T. Yoshioka (Natl. Inst. Cro. Sci, NARO) Difference of discoloration of boiled grain among proanthocyanidin-free barley NILs.

P3. Konishi, S., T. Sasanuma, T. Sasakuma (Kihara Inst. Biol. Res., Yokohama City U.) Organ-specific expression of *Mlo* gene family in *Triticum aestivum*.

P4. Terasawa, Y.[1], T. Sasanuma[1], T. Kawahara[2], T. Sasakuma[1] (1. Kihara Inst. Biol. Res., Yokohama City U., 2. Grad.Sch. Agr., Kyoto U.) Diversity of the HMW-glutenin subunits of wheat landraces in Afghanistan, Iran and Pakistan collected in 1955, 1965 and 1979.

P5. Tamura, T., T. Sasanuma, T. Sasakuma (Kihara Inst. Biol. Res., Yokohama City U.) Isolation of *vrn1* and *2* homologs in *Aegilops umbellulata*.

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P9. Shitsukawa, N.[1], A. Takagishi[1], C. Tahira[1], S. Takumi[2], K. Murai[1] (1. Dep. Biosci., Fukui Pref. U., 2. Fac. Ari., Kobe U.) The molecular basis of wheat spike formation.

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Others

Editorial Remarks

We are pleased to announce the publication of the 101st issue of Wheat Information Service.

Wheat Information Service (WIS) was founded in 1954 as an international newsletter for wheat geneticists and breeders. As mentioned in the "Important Announcement on WIS – Discontinuance of Publication" by Dr. Kozo Nishikawa, the Editor-in-Chief, the prime aim of WIS was to promote "exchanging information about wheat genetics and breeding among wheat researchers in the world" (No. 98, 2004). In December 2005, WIS published the No. 100 commemorative issue and put a period to its five-decade long activity.

In appreciation of the significance of WIS as an accessible media for wheat researchers worldwide, the Japanese wheat geneticist community decided to launch "Wheat Information Service – electronic newsletter for wheat researchers" (namely, eWIS) as a new World-Wide-Web-based newsletter for informal research information circulation. Five scientists voluntarily participated in the new Editorial Office and worked together to set up the brand-new newsletter.

To launch eWIS, we reviewed thoroughly the editorial guidelines of the original WIS and made a number of important revisions. Details of the new editorial guidelines are provided in the Instructions to Authors. Of all those revisions, we would like to call special attention to the followings. Firstly, eWIS is published online only and distributed through the eWIS web page (http://www.shigen.nig.ac.jp/ewis). No hard-copy edition will be supplied. Secondly, electronic submission is mandatory. Hard-copy manuscripts will not be considered for publication. Thirdly, manuscripts will no longer be peer-reviewed for publication, while the Editorial Office reserves the right to make final decision for acceptance. Submitted manuscripts will be checked for the contents and style prior to publication. However, the authors should note well that the Editorial Office is not organized to deal with articles that constitute formal publications. Fourthly, it is now a policy that citations to the eWIS articles should be accompanied by permission from the authors. The data and ideas published in eWIS are made available by their authors with the understanding that they will not be used in publications without their specific consent.

Despite all those changes, eWIS continues to stand with the aim of the original WIS. eWIS is designed to serve the wheat community as an informal, rapid communicator. Considering marked progress in plant sciences, there is a great need to share test results, technical tips, protocols, mutant and germplasm descriptions, and genetic map information that may be useful in the lab and field. We hope that eWIS will provide a use-friendly media to circulate private knowledge that might otherwise go unrecorded.

eWIS welcomed seven wheat researchers as Advisory Board members to whom we are grateful for their valuable advices and encouraging comments. The eWIS web site and electronic submission system were set up by two system specialists, Kazuhiro Oogushi and Takehiro Yamakawa in the Dr. Yukiko Yamazaki's lab (National Institute of Genetics, Japan). We acknowledge their great assistance. The Editorial Office was partly supported by a Grant-in-Aid for the National Bioresource Project of the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

February 2006

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Instructions to Authors

eWIS welcomes manuscripts that provide test results, technical tips, protocols, mutant and germplasm descriptions, map information, and any other information that may be useful in the lab and field. The articles are informal, non-peer-reviewed, thus do not constitute formal publications. Only manuscripts that require minimal editing will be considered for publication.

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The manuscript should start with a title, the names of author(s), affiliation(s), abstract, followed by the text. Abstract may be omitted if not necessary. Do not divide the text into subsections, such as M&M, Results and Discussion. There is no fixed limit on the length but a concise presentation is encouraged.

(2) **Research Opinion & Topics**: Reviews, mini-reviews, trends and topics in wheat research.

Authors who wish to submit a (mini-)review should contact the Editorial Office prior to submission.

(3) **Meeting Reports**: Announcement of forthcoming meeting and reports on the meeting attended

(4) **Others**: Any other information useful for wheat researchers

Title, Affiliation and Abstract

In the title page(s), the manuscript category (as mentioned above), a title, the names of the author(s), affiliation(s) and address(es) of the authors, and the e-mail address, telephone, and fax numbers of the corresponding author must be clearly indicated.

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Payne PI, Holt LM, Law CN (1981) Structural and genetical studies on the high molecular weight subunits of wheat glutenin. Theor Appl Genet 60:229-236.

Book chapters:

Peacock WJ, Dennis ES, Gerlach WJ (1981) Molecular aspects of wheat evolution: repeated DNA sequences. In: Evans LT and Peacock WJ (eds.) Wheat Science - Today and Tomorrow. Cambridge Univ. Press, Cambridge, UK, pp. 41-60.

Books:

Knott DR (1989) The Wheat Rusts - Breeding for Rust Resistance. Springer-Verlag, New York, USA.

Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., K. Tsunewaki personal communication).

Abbreviations
Abbreviations should be explained at first occurrence.

Symbols and Units
Gene names and protein names must carefully be discriminated. Gene names and loci should be italicized; protein should be upright. The SI units (http:// physics.nist.gov/Pubs/SP330/contents.html) should be used throughout.

Nomenclature
Nomenclature of genes and chromosomes should follow the 'Catalogue of gene symbols for wheat' (McIntosh *et al.*: 10th Int. Wheat Genet. Symp. 2003).

Nucleotide sequences
The DDBJ/EMBL/GenBank accession numbers must be provided for newly reported nucleotide sequences.

Tables
Tables must be numbered consecutively. For Table writing, Microsoft Word is recommended. Prepare a separate file for each table. Refer to the latest eWIS articles for format.

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Authors of accepted manuscripts are informed by e-mail that a temporary URL has been created from which they can obtain their proof. Proofreading is the responsibility of the author. Authors should make proof corrections and send them to Editorial Office by e-mail. After online publication, corrections can only be made in exceptional cases when Editorial Office permits the necessity.

The final version of accepted manuscripts will be published in the 'Online First' section of the eWIS web page upon receipt of proof corrections. Editorial Office biannually gathers the accepted manuscripts published in the 'Online First' into a volume. In 'Archive' of eWIS, all manuscripts are collected as PDF format, and open to all wheat researchers.

No hard-copy edition will be supplied. For each volume, a PDF edition will be available for downloading.

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