



# Wheat Information Service

## Electronic Newsletter for Wheat Researchers

No. 105

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## Research Information

# Coleoptile color variation in *Aegilops tauschii* Coss. II. The Caucasus, Turkey, and Syria

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I report coleoptile color variation observed in *Aegilops tauschii* accessions collected in the Caucasus, Turkey, and Syria. Sixty-eight accessions were used in this study (Table 1). These accessions were the collections of the Centre for Genetic Resources (CGN accessions), Institut für Pflanzengenetik und Kulturpflanzenforschung (AE accessions), International Center for Agricultural Research in the Dry Areas (IG accessions), Plant Germ-plasm Institute (KU accessions), and United States Department of Agriculture (PI accessions). In December of 2003 and 2004, seeds (one per accession) were sown and the plants grown individually in pots in a slightly-heated

greenhouse at Fukui Prefectural University. Coleoptile color was checked by eye and recorded as red (i.e., anthocyanin pigmentation) or green (i.e., no anthocyanin pigmentation). One accession (IG 127015, Armenia) had green coleoptile, whereas all the others had red coleoptile (Table 1). I thank H. Bockelman (PI accessions), A. Graner (AE accessions), T. Kawahara (KU accessions), J. Konopka (IG accessions), J. Valkoun (IG accessions), and L. Visser (CGN accessions) for the seeds.

**Table 1. Coleoptile color of *Ae. tauschii* accessions collected in the Caucasus, Turkey, and Syria**

No.	Accession No.	Country	Locality	Coleoptile color
1	CGN 10734	Armenia	-	Red
2	IG 47173	Armenia	Tech town, Kafan	Red
3	IG 48747	Armenia	Muchavan NE Erevan, Abovyan	Red
4	IG 48748	Armenia	Garni, Abovyan	Red
5	IG 48758	Armenia	Shorap, Ashtarak	Red
6	IG 126273	Armenia	near Urtsadzor village, Ararat	Red
7	IG 126280	Armenia	1.5 km from v. Shagat to E, Ararat	Red
8	IG 126293	Armenia	Getup village, Ehegnadzorski	Red
9	IG 126353	Armenia	Sof Kechut, Vanadzor	Red
10	IG 126991	Armenia	Kapan distr., Sjunik	Red
11	IG 127015	Armenia	Megri distr., Sjunik	Green
12	KU-2809	Armenia	Cytoanka Park, Erevan	Red
13	KU-2810	Armenia	Cytoanka Park, Erevan	Red
14	KU-2811	Armenia	Cytoanka Park, Erevan	Red
15	KU-2814	Armenia	Cytoanka Park, Erevan	Red

16	KU-2816	Armenia	Aragaband (Erevan - Airport)	Red
17	KU-2821	Armenia	14 km E of Erevan (Eravan - Garni)	Red
18	KU-2822A	Armenia	3 km S of Bjurakan (Eravan - Bjurakan)	Red
19	KU-2823	Armenia	1 km S of Bjurakan (Eravan - Bjurakan)	Red
20	KU-2824	Armenia	1 km S of Bjurakan (Eravan - Bjurakan)	Red
21	CGN 10731	Azerbaijan	-	Red
22	CGN 10732	Azerbaijan	-	Red
23	IG 47182	Azerbaijan	Alvadee village, Masallinski	Red
24	IG 47186	Azerbaijan	Caily village, Shemakha	Red
25	IG 47188	Azerbaijan	Getgashena, Kutkashen region	Red
26	IG 47192	Azerbaijan	Road to Yardymly	Red
27	IG 47193	Azerbaijan	Palish, Lerik region	Red
28	IG 47194	Azerbaijan	Apsheron region	Red
29	IG 47196	Azerbaijan	Hippodrome, Baku	Red
30	IG 47199	Azerbaijan	Geoglyarskaya road, Shemakha region	Red
31	IG 47202	Azerbaijan	Dagdagan town, Stephanakert region	Red
32	IG 47203	Azerbaijan	Granitsi-Fizuli, Dzhebrailisk region	Red
33	IG 47204	Azerbaijan	Bilidzi town, Divichi	Red
34	KU-2801	Azerbaijan	Sumgait, 34 km N of Baku	Red
35	KU-2804	Azerbaijan	10 km E of Shemakha (Baku - Shemakha)	Red
36	KU-2806	Azerbaijan	10 km E of Shemakha (Baku - Shemakha)	Red
37	IG 48274	Dagestan	Upper Dzhalgan village	Red
38	IG 120863	Dagestan	Magaramkent (53 km S of Derbent)	Red
39	IG 120866	Dagestan	Frig, 2 km of Belidzhi (30km S of Derbent)	Red
40	KU-20-1	Dagestan	Derbent	Red
41	AE 454	Georgia	Mzcheta	Red
42	AE 457	Georgia	Teleti	Red
43	AE 929	Georgia	Mccheta Dzvari	Red
44	AE 933	Georgia	Mccheta-Tbilisi	Red
45	AE 1037	Georgia	20 km NW Citeli-Ckaro	Red
46	KU-2826	Georgia	35 km SSE of Tbilisi (Erevan - Tbilisi)	Red
47	KU-2827	Georgia	Suburbs of Tbilisi (Botanical Garden)	Red
48	KU-2828	Georgia	Lake Tbilisi, Tbilisi	Red
49	KU-2829A	Georgia	Suburbs of Tbilisi, NW of Tbilisi	Red
50	KU-2832	Georgia	Suburbs of Tbilisi, NW of Tbilisi	Red
51	KU-2834	Georgia	Suburbs of Tbilisi, NW of Tbilisi	Red
52	KU-2835B	Georgia	59 km NW of Tbilisi (Tbilisi - Gori)	Red
53	KU-2836	Georgia	6 km NE of Gori (Tbilisi - Gori)	Red
54	IG 46623	Syria	63 km from Al Hasakah to Ra's al 'Ayn	Red

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55	IG 47259	Syria	12 km S Rasafa	Red
56	KU-2131	Turkey	24.7 km SW from Van to Tatvan	Red
57	KU-2132	Turkey	24.7 km SW from Van to Tatvan	Red
58	KU-2133	Turkey	32.2 km NE from Van to Ozalp	Red
59	KU-2136	Turkey	46.1 km N from Van to Ercis	Red
60	KU-2137	Turkey	64.7 km NNE from Van to Ercis	Red
61	KU-2138	Turkey	76.7 km NNE from Van to Ercis	Red
62	KU-2140	Turkey	76.7 km NNE from Van to Ercis	Red
63	KU-2141	Turkey	87.6 km N from Van to Ercis	Red
64	PI 486267	Turkey	6km S of Semdinli, Hakkari	Red
65	PI 486270	Turkey	37km NE of Yuksekova, Hakkari	Red
66	PI 486274	Turkey	35km W of Tuzluca, Kars	Red
67	PI 486277	Turkey	24km SW of Kagizman-Igdir-Erzurum Road Junction, Kars	Red
68	PI 554319	Turkey	12 km SW of Hakkari on road to Uludere, Hakkari	Red

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## Research Information

# Are rye centromeric repetitive sequences confined in the centromere of rye chromosomes?

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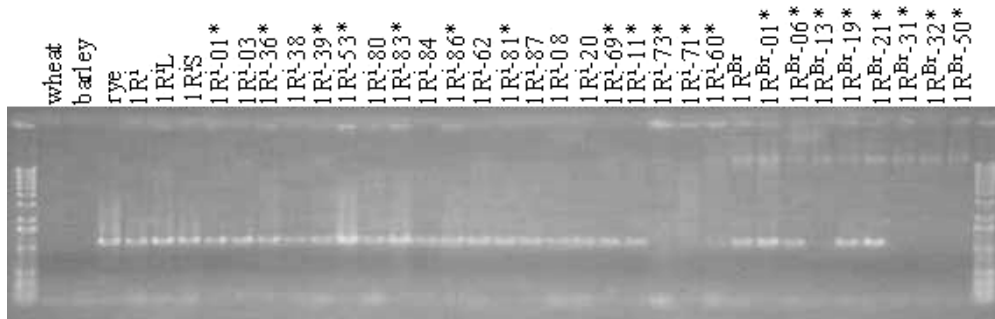
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Tsuchida et al. (2008) cytologically dissected rye chromosome 1R in common wheat by the gametocidal system and established 55 common wheat lines carrying structurally rearranged 1R chromosomes, such as terminal deletions and translocations between 1R and wheat chromosomes. They also reported that seven of the 55 lines carry Robertsonian translocations between 1R and wheat. However, it is difficult to draw a distinction between the breakages in the centromere and those in the pericentromeric regions by microscopy. Therefore, it was not known which centromere, that of rye or that of wheat or both, those translocations chromosomes had.

Francki (2001) isolated and characterized a rye centromeric repetitive element and showed by FISH that the sequence (3.4 kbp repetitive sequence, pAWRC. 1), representing a diverged family of retrotransposon-like elements, called the *Bilby* family, localized within the centromeres of all rye chromosomes and no signal was detected on wheat chromosomes. By using the rye centromeric repeat, we attempted to elucidate the centromere identity of the rearranged 1R chromosomes whose breakpoints were thought to be located in the centromere. We used 20

1R<sup>i</sup> dissection lines developed by Tsuchida et al. (2008). Eight of them had telocentric chromosomes and 12 lines carried translocations between 1R<sup>i</sup> and wheat chromosomes including: The translocations in seven lines were of Robertsonian type and those in the remaining five lines carried 1R<sup>i</sup>L segments translocated onto wheat chromosomes, which were used as negative controls (Table 1). We also employed euploid rye 'Imperial', and 1R<sup>i</sup>, 1R<sup>i</sup>L and 1R<sup>i</sup>S addition lines of common wheat 'Chinese Spring' as positive controls, and euploid barley 'Betzes'. These lines were obtained from National BioResource Project-Wheat, Japan (<http://www.shigen.nig.ac.jp/wheat/komugi/top/top.jsp>). In addition, we used eight dissection lines of another 1R chromosome derived from a wheat cultivar Burgas 2 with a 1R (1B) substitution (designated 1R<sup>Br</sup> in this paper) together with a positive control of 1R<sup>Br</sup> addition line of common wheat (Table 1, unpublished).

We designed a primer pair from the sequence of pAWRC. 1 and conducted PCR analysis. The PCR amplification of pAWRC. 1 was seen in all 15 1R<sup>i</sup> dissection lines carrying rearranged 1R<sup>i</sup> chromosomes with breakpoints in the centromere, four out of the

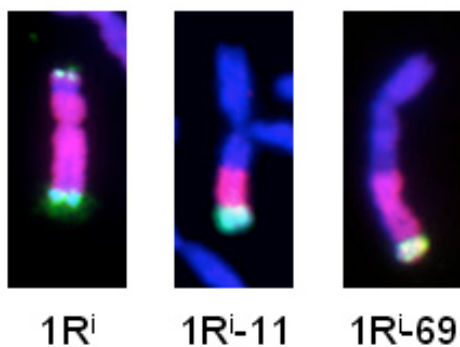


**Figure 1.** Amplification of the PCR marker, pAWRC. 1, in the 1R<sup>i</sup> and 1R<sup>Br</sup> dissection lines, and in the positive and negative control lines. The 1R<sup>i</sup>, 1R<sup>i</sup>L, 1R<sup>i</sup>S and 1R<sup>Br</sup> lines had normal 1R chromosome of Imperial rye, its long arm, its short arm and normal 1R chromosome derived from a common wheat cultivar Burgas 2. For the structural characteristics of the dissection lines, see Table 1. Single asterisks indicate that the rearranged chromosomes are translocations, and the others are deletions.

**Table 1. Rearranged 1R chromosomes and the presence or absence of the rye-specific centromeric repetitive sequence pAWC.1**

Line	Type of rearrangement	Breakpoint*	pAWRC.1
wheat	—	—	absent
Barley	—	—	absent
Rye	—	—	present
1R <sup>i</sup>	—	—	present
1R <sup>i</sup> L	—	—	present
1R <sup>i</sup> S	—	—	present
1R <sup>i</sup> -01	translocation	centromere (l)	present
1R <sup>i</sup> -03	deletion	centromere (l)	present
1R <sup>i</sup> -08	deletion	long arm/centromere	present
1R <sup>i</sup> -11	translocation	long arm (wc)	present
1R <sup>i</sup> -20	deletion	satellite/centromere	present
1R <sup>i</sup> -36	translocation	centromere (l)	present
1R <sup>i</sup> -38	deletion	centromere (l)	present
1R <sup>i</sup> -39	translocation	centromere (l)	present
1R <sup>i</sup> -53	translocation	centromere (l)	present
1R <sup>i</sup> -60	translocation	long arm/long arm (wc)	absent
1R <sup>i</sup> -62	deletion	centromere (s)	present
1R <sup>i</sup> -69	translocation	long arm (wc)	present
1R <sup>i</sup> -71	translocation	long arm (wc)	absent
1R <sup>i</sup> -73	translocation	long arm (wc)	absent
1R <sup>i</sup> -80	deletion	centromere (l)	present
1R <sup>i</sup> -81	translocation	centromere (s)	present
1R <sup>i</sup> -83	translocation	centromere (l)	present
1R <sup>i</sup> -84	deletion	centromere (l)	present
1R <sup>i</sup> -86	translocation	centromere (l)	present
1R <sup>i</sup> -87	deletion	centromere (s)	present
1R <sup>Br</sup>	—	—	present
1R <sup>Br</sup> -01	translocation	centromere (l)	present
1R <sup>Br</sup> -06	translocation	centromere (l)	present
1R <sup>Br</sup> -13	translocation	centromere (s)	absent
1R <sup>Br</sup> -19	translocation	centromere (l)	present
1R <sup>Br</sup> -21	translocation	centromere (l)	present
1R <sup>Br</sup> -31	translocation	centromere (s)	absent
1R <sup>Br</sup> -32	translocation	centromere (s)	absent
1R <sup>Br</sup> -50	translocation	centromere (l)	absent

\* (l) and (s) indicate that the rearranged 1R chromosomes with breakpoints in the centromere had the 1R long arm and 1R short arm, respectively. (wc) represent that the translocated 1R chromosomes had wheat centromere.



**Figure 2.** GISH and FISH (probe: pSc200) images of the normal 1R<sup>i</sup> chromosome and rearranged chromosomes 1R<sup>i</sup>-11 and 1R<sup>i</sup>-69. Note that the centromeres of 1R<sup>i</sup>-11 and 1R<sup>i</sup>-69 are of the wheat ones.

eight 1R<sup>Br</sup> dissection lines and all positive controls (Table 1, Fig. 1). This result suggested that those 15 rearranged 1R<sup>i</sup> and four rearranged 1R<sup>Br</sup> chromosomes had their breakpoints in the middle of the centromeric region and that the other four rearranged 1R<sup>Br</sup> chromosomes had their breakpoints in the vicinity of, but not in, the centromere. As expected, pAWRC. 1 was not amplified in wheat and barley, and three 1R<sup>i</sup> dissection lines 1R<sup>i</sup>-73 and 1R<sup>i</sup>-71, and 1R<sup>i</sup>-60, but unexpectedly, it was amplified in 1R<sup>i</sup>-11 and 1R<sup>i</sup>-69 (Table 1, Fig. 2). Judging from the FISH/GISH images (Fig. 2) and from the PCR analysis by Tsuchida et al. (2008), it is obvious that these two lines did not have the rye centromere: the 1R<sup>i</sup>-11 line lacked all PCR markers on the 1R<sup>i</sup> short arm and two proximal PCR markers on the 1R<sup>i</sup> long arm; the 1R<sup>i</sup>-69 line lacked all PCR markers on the 1R<sup>i</sup> short arm and one proximal PCR markers on the 1R<sup>i</sup> long arm. In these lines there may have been rye centromere segments that were too small to be detected by cytological observation. If this is the case, we may be able to exploit such minichromosomes to create artificial chromosomes by a top-down approach (see a review, Houben and Schubert 2007). The other possibility is that the 1R<sup>i</sup> long arm contained the same centromeric sequences in some region other than the centromere. If this is the case, it might be said the long arm of 1R<sup>i</sup> was originated from a complicated structural rearrangements, like a pericentromeric inversion involving the centromeric sequences. We are planning to elucidate which is the case by examining the segregating progeny of the 1R<sup>i</sup>-69 and 1R<sup>i</sup>-11 lines that were hemizygous for the critical rearranged 1R<sup>i</sup> chromosomes.

Generally, it is difficult to prove that so-called

centromere-specific sequences are really confined to the centromere or the primary constriction. Molecular analysis is highly sensitive to detect the presence of certain centromeric sequences but it is helpless to tell the chromosomal location of such sequences. At present *in situ* hybridization is the only way to know the chromosomal location of centromeric sequences. It is a powerful tool when such centromeric sequences are concentrated in a chromosomal region, e.g. the primary constriction but otherwise it is also helpless to detect dispersed centromeric sequences. Combined with PCR analysis, rye-wheat translocation chromosomes, like 1R<sup>i</sup>-69 and 1R<sup>i</sup>-11, that have the wheat centromeres and rye chromosomal segments are useful in proving whether or not candidate sequences for the rye centromere are really confined to the centromere. Whatever the case may be, the result of the study we are planning to start would bring a fascinating development in the study of the cereal centromere structure.

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## Topics on Wheat Genetic Resources

# National Bioresources Project-Wheat

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In 2002, the Japanese government launched the National Bioresource Project (NBRP) in order to promote scientific researches collectively not only in Japan but also in the world. The aims of NBRP are the strategic propagation, maintenance, and distribution of living organisms, cells and DNA clones of major plant and animal species, one of which is wheat. NBRP-Wheat (Komugi in Japanese) was originally assigned to a core group consisting of the Graduate School of Agriculture, Kyoto University (LPGKU, MOZUME), Kihara Institute for Biological Research, Yokohama City University (KIBR), and the Faculty of Agriculture, Tottori University (TACBOW). Researchers in Japan conducting wheat studies form a committee to support the core group.

NBRP-Wheat is in charge of wheat (genus *Triticum* and genus *Aegilops*) and other related species, rye (genus *Secale*) and oats (genus *Avena*). Plant strains collected are being examined for morphological and genomic characters before seed propagation and conservation. During the first stage of NBRP-Wheat, 2002-2006, more than 7000 wheat stocks, including wild species, landraces and genetic stocks, have been collected, propagated and conserved. Also, more than a million genomic DNA and EST clones have

been collected and conserved, and more than a half million ESTs have been analyzed. All data is stored in the NBRP section of the database KOMUGI. The registered seed stocks and DNA clones are available through the internet service (<http://www.shigen.nig.ac.jp/wheat/komugi/top/top.jsp>).

The Laboratory of Plant Genetics, Kyoto University (LPGKU) is in charge of the collection of various seed stocks of wheat (*Triticum*) that were produced in the past during genetic studies on wheat. These stocks have been propagated after cytological examination. The following seed stocks (642 as of November 2007) have been collected at LPGKU and registered at the database KOMUGI for wide distribution.

In 2007, the second stage of NBRP-Wheat has started. In the second stage, in addition to maintain and to distribute the genetic stocks collected during the first stage of NBRP-Wheat, we are focusing on collecting polymorphic DNA markers that would be useful in genetic studies and wheat breeding. The second stage of NBRP-Wheat is planned to continue until 2012 to achieve the best collection of wheat genetic stocks in the world.

**Table 1. Collection in LPGUK**

Strain name	Count
Aneuploid lines of common wheat ( <i>Triticum aestivum</i> ) cv. Chinese Spring	126
Monosomics (2n=41, 20"+1')	21
Ditelosomics (2n=42, 20"+1t")	42
Doubleditelosomics (2n=44, 20"+1t"+1t")	21
Nullisomic-Tetrasomics (2n=42, 1 <sup>iv</sup> +19")	42
Aneuploids of other common wheat	42
Norin 61 Monosomics (2n=41, 20"+1')	21
Norin 26 Monosomics (2n=41, 20"+1')	21

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Deletion lines of common wheat cv. Chinese Spring	348
Group5	53
Group6	44
Group7	65
Group3	39
Group1	57
Group4	45
Group2	45
Alien addition and substitution lines	41
Barley addition lines	21
Gametocidal chromosome addition lines	20
Alien chromosome dissection lines	23
Barley 5H dissection lines	23
Alien cytoplasm substitution lines	47
Chinese Spring alloplasmic lines	47
Aneuploid lines of durum wheat ( <i>Triticum turgidum</i> )	15
Langdon durum D-genome substitution lines	15

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## Topics on Wheat Genetic Resources

# A report of the work in National Bioresource Project-Wheat in Tottori University: Wheat lines with alien chromosome

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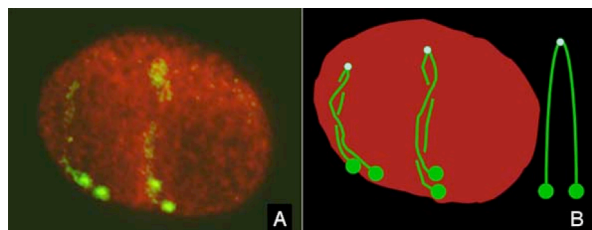
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In long history of genetical studies of wheat, researchers have produced many interspecific or intergeneric hybrids and amphidiploids (Maan and Gordon 1988). These plants were further used as the source of wheat lines with a pair of alien chromosomes called 'alien chromosome addition lines' (Shepherd and Islam 1988). These plants are valuable to introduce useful genes to wheat from the other species (Friebe et al. 1996). Many of the lines have been incorporated in practical breeding programs. These lines are also interesting for basic genetical studies. For example, cytological analysis with genomic *in situ* hybridization (GISH) reveals the behavior of the chromosomes in interphase nuclei (Fig. 1).

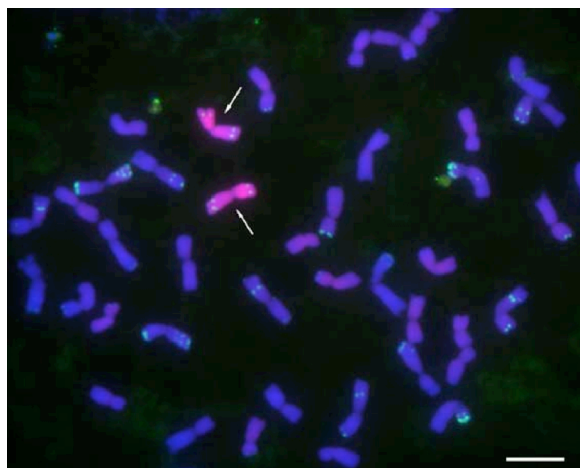
In 2003 we joined the National Bioresource Project-Wheat. We re-evaluated the chromosomes, propagated fresh seeds and stored them in cold and dry room (Fig. 2, Table 1). Some of the seeds were distributed to researchers. The lines were classified into several groups to make users access easily to the appropriate lines in the database (Table 2). We could successfully propagate many of the lines. However, we failed to do in some strains because of severe sterility, no germination, or instability or absence of

expected alien chromosomes. This year the stocks were transferred from Tottori University to Kyoto University for centralized control following advice by the government. The service of material distribution will be continued from Kyoto University.

Most of the present common wheat is the derivatives of a single or a limited numbers of interspecific hybrids between tetraploid wheat and *Aegilops tauschii* that appeared mere 8,000 years ago. All variations in common wheat are thus those accumulated in such short period of the evolution. To expand variation of common wheat for future demand, genes of the other species are indispensable. However,



**Figure 1.** Genomic *in situ* hybridization to a nucleus of the *Leymus racemosus* J chromosome addition line (TACBOW0007). Chromosome J are recognized even in interphase nucleus.



**Figure 2.** FISH/GISH image of a line in NBRP-Komugi bank as an example (TACBOW0041 *Agropyron elongatum* 4E addition). All alien lines are evaluated by FISH, GISH and/or C-banding methods (Red: probe of *Ag. elongatum* DNA, green: probe of 120-bp family sequence).

**Table 1. Numbers and types of genetic experimental lines of wheat handled in Tottori University**

Category no.*	Type	No. of accessions
S1	Wild species	5
S2	Landrace/Cultivar/Breeding line	41
T	Other genera in Triticeae**	11
W3.6	Synthetic polyploid	144
W.3.7.8	Alien chromosome addition or substitution lines	178

\* The materials handled in NBRP-Wheat were classified into several groups (Table 2).

\*\* Triticeae genera other than *Triticum* and *Aegilops*.

**Table 2. Classification of the genetic stocks for easy access**

Type	Category no.
Triticeae	
<i>Triticum</i> and <i>Aegilops</i>	
Wild species	
<i>Triticum</i>	W1.1
<i>Aegilops</i>	W1.2
Landrace/Cultivar/Breeders line	W2
Experimental line	
Mutant	W3.1
Isogenic line	W3.2
Tester	W3.3
Recombinant inbred line	W3.4
Intervarietal chromosome substitution line	W3.5
Synthetic polyploid	W3.6
Aneuploid	
Nullisomics	W3.7.1
Monosomics	W3.7.2
Tetrasomics	W3.7.3
Nulli-tetrasomics	W3.7.4
Telocentrics	W3.7.5
Deletion line	W3.7.6
Other	W3.7.7
Alien chromosome addition and substitution line	W3.7.8
Alien chromosome deletion and translocation line	W3.7.9
Cytoplasmic substitution lines	W3.8
Transformant	W4
Others	W5
<i>Secale</i>	
Wild species	S1
Landrace/Cultivar/Breeders line	S2
Other genera in Triticeae	T
Oat	
<i>Avena</i>	
Wild species	A1
Landrace/Cultivar/Breeding line	A2
Chromosome and genome line	A3
Alien chromosome and genome line	A4
Others	A5

other species, especially wild species, are not able to be evaluated as 'wheat' because of great morphological difference from wheat. Once promising gene is discovered in such species by molecular survey, the wheat line carrying the chromosome with the gene would have great value to demonstrate the phenotype. Connection of the genetical studies to breeding programs will have an impact on world agriculture.

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Topics on Wheat Genetic Resources

## The report of National Bioresource Project: Seed resources of the Plant Germ-plasm Institute, Graduate School of Agriculture, Kyoto University

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In the first stage (2002-2006) of National BioResource Project (NBRP), Laboratory of Crop Evolution, Plant Germ-plasm Institute (PGPI), has acted as a center responsible for the conservation and multiplication of wheat landraces and wheat relatives including wild *Aegilops* and *Triticum* species. During the five years of the first stage, a total of 3,824 accessions of wheat and its relatives, consisting of 1,305 accessions of landraces, 394 of wild wheats, 1,707 of wild *Aegilops* and 418 of miscellaneous *Triticum* and *Aegilops* species, were multiplied in PGPI and stored as a genetic resource of NBRP (Table 1). Passport data of accessions maintained at PGPI were checked and revised catalogue was published in 2005 (Kawahara 2005).

In addition to the conservation and multiplication program, preliminary survey of physiological traits and molecular genotyping were carried out for the purpose of efficient use of these genetic resources. Growth habit of diploid *Aegilops* and *Triticum* species were checked under continuous light in a heated glasshouse (minimum of 15 degrees centigrade) together with several standard lines of Japanese wheat cultivars. In this condition, spring cultivars extended their flag leaves within 50 days but winter cultivars needed more than 100 days for flag leaf extension. Therefore we classified accessions that extended their flag as leaves within 50 days “spring”, those needed more than 100 days as “winter” and those in-between as “intermediate” (Table 2; Figure 1). Most accessions of diploid wheat and *Ae. tauschii* were winter type but several spring accessions were found in *Ae. tauschii*. While, majority of *Ae. unbellulata* accessions were intermediate and many spring accessions were found in this species.

We also analyzed intra- and interspecific variation in chloroplast DNA (cpDNA) in diploid species in

**Table 1. List of NBRP genetic resources multiplied in PGPI**

Category	Number of accessions
Wheat landraces	1,305
<i>Triticum aestivum</i>	1,114
<i>T. durum</i>	172
<i>T. monococcum</i>	19
Wild wheats	394
<i>T. araraticum</i>	335
<i>T. boeoticum</i>	59
<i>Aegilops</i> species	1,707
<i>Ae. biuncialis</i>	135
<i>Ae. caudata</i>	274
<i>Ae. columnaris</i>	49
<i>Ae. cylindrica</i>	60
<i>Ae. mutica</i>	42
<i>Ae. ovata</i>	132
<i>Ae. speltoides</i>	231
<i>Ae. tauschii</i>	88
<i>Ae. triaristata</i>	189
<i>Ae. triuncialis</i>	296
section <i>Comopyrum</i> species	52
subsection <i>Emarginata</i> species	90
section <i>Vertebrata</i> polyploid species	69
Others	418
<i>Triticum</i> species	217
<i>Aegilops</i> species	201
Total	3,824

**Table 2. Growth habit of diploid *Aegilops* and *Triticum* species**

Category (genome)	Year	Spring*	Intermediate	Winter	Total
Diploid wheat (AA)	2004/2005	0 (0.0)	105 (37.8)	173 (62.2)	278
<i>Ae. tauschii</i> (DD)	2005	4 (1.9)	40 (19.0)	166 (79.0)	210
<i>Ae. umbellulata</i> (UU)	2006	24 (14.6)	98 (59.8)	42 (25.6)	164

\* for details, see text.



**Figure 1.** Screening of growth habit in Plant Germ-plasm Institute (February 2005, Muko, Japan).

order to obtain basic information for species identification by DNA sequence (Yamane and Kawahara 2005, Yamane et al. 2006). These analyses focused on DNA sequence variation in noncoding regions of cpDNA, which included base-pair substitutions, insertion/deletions, microsatellites, and inversions. Nine of 13 *Aegilops-Triticum* species were successfully identified and genotyped using these data. Sixty-two haplotypes were detected in 115 accessions of 13 diploid species. Because of the large number of characters examined, novel deep relationships within

and among *Aegilops-Triticum* species could be identified and evaluated.

Furthermore, in collaboration with this NBRP project, we started to construct core collection of *Ae. tauschii*. By chloroplast DNA fingerprinting, about 450 *tauschii* accessions with well-documented collection sites from major genebanks are already evaluated.

The production of *Tauschii* Core Collection will facilitate the utilization of the available genetic resources for the future wheat breeding.

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## Topics on Wheat Genetic Resources

# The report of the work of National Bioresource Project: Seed resources of Kihara Institute for Biological Research

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In the first stage of National BioResource Project (NBRP), Kihara Institute for Biological Research (KIBR) has acted as a subcenter responsible for the conservation and multiplication of wheat “experimental lines” and “landraces”. During the five years of the first stage (2002-2006), a total of 1,849 accessions of wheat and its relatives, in which 428 accessions of experimental lines and 1,154 accessions of landraces were included, were multiplied in KIBR and stored as a genetic resource of NBRP (Table 1 and Figure 1).

The experimental lines include mutant lines, chromosome substitution lines, near isogenic lines (NILs), and recombinant inbred lines (RILs). These kinds of lines, especially the NILs and RILs, are powerful materials for genetic analyses, but the successive backcrossing or selfing for more than ten years were required to produce such lines, and once lost it is difficult to recover them. Therefore, the stable and authorized support of NBRP to the work of maintenance and supply of such materials is valuable and helpful for wheat researchers.

**Table 1. List of NBRP wheat genetic resources multiplied in KIBR**

Category	Number of accessions
Experimental lines	428
Diploid RILs	114
Diploid mutant lines	32
Hexaploid RILs	147
Hexaploid mutant lines	47
Hexaploid NILs	26
Hexaploid chromosome substitution lines	62
Landraces	1,154
Diploid ( <i>Triticum monococcum</i> )	13
Hexaploid other than <i>T. aestivum</i> ( <i>T. spelta</i> , etc.)	12
Hexaploid in Afghanistan*	444
Hexaploid in Uighur	340
Hexaploid in Tibet	345
Others	267
Diploid wild species	7
Hexaploid other cultivars	260
Total	1,849

\*Afghan landraces have been maintained in Germ-Plasm Institute, Kyoto University. KIBR was commissioned the multiplication throughout NBRP.



**Figure 1.** A picture of wheat field in KIBR, where Afghan wheat landraces were growing (May 2004, Yokohama, Japan).

Landraces are the traditional cultivars, which have been grown in a local area for a long period and responsible for the regional climate and cultures. With respect to the agronomical traits such as grain yield and bread making quality, landraces are inferior to modern cultivars. The landraces, however, are thought to possess adaptation genes against environmental stress, such as drought or cold tolerant and disease resistant genes. As known well, the bread wheat is hexaploid originated in the hybridization between cultivated tetraploid wheat and wild diploid goatgrass (Kihara 1944, McFadden and Sears 1946). The characteristic of hybrid origin and hexaploidy gives the bread wheat several problems for breeding, that is, the lack of stably crossable wild species and difficulty of producing the drastically expressed mutation. From these reasons, the expansion of genetic resource of bread wheat is more difficult than other major cereal crops, such as rice and barley. Therefore, the hexaploid landraces, which have been grown under a

strong selection of severe local climate and ethnic culture and consequently has come to contain a wide range of genetic variation, are considered to be the most important primary genetic resources of bread wheat.

In the NBRP program, KIBR multiplied and stored wheat landraces of Afghanistan, Uighur and Tibet, which were collected by successive explorations, as represented by Dr. Hitoshi Kihara's historical expedition called KUSE (Yamashita 1965). In addition to the conservation and multiplication program, systematic survey of qualitative and quantitative morphological traits and molecular genotyping are being carried out for the purpose of using the genetic resources efficiently and applicatively. Furthermore, for the next stage, several accessions are selected from the large number of accessions based on the morphological and molecular data as representative collections, which are named "core collection". The construction and utilization of core collection is progressing and more detailed data on our genetic resources is accumulating, which will certainly improve the scientific quality of our materials.

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Topics on Wheat Genetic Resources

# National BioResource Project of Japan: DNA resource of Wheat

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Wheat is characteristic of its polyploid nature. Mainly because of its huge genome size (5Gbp per genome), information of entire genome sequencing is not sufficient. In order to carry out functional genomics in common wheat, we had made a plan to complete expressed sequence tags (ESTs) in common wheat mainly using Chinese Spring for construction of gene expression map in various tissues of wheat and supplying new marker information for the chromosome mapping. At first, we extracted total RNAs from 11 tissues during the wheat life cycle. The cDNA libraries with plasmid had been constructed from these RNAs without any amplification, so that the cDNA libraries should be reflected their mRNA

abundances. After several thousands bacterial colonies from each library were randomly picked up, one pass sequencing had been carried out from both ends of each insert DNA. These ESTs were classified into certain contigs with the phrap method. We were able to distinguish each contig corresponding to their homoeologues from three genomes, namely A, B and D by adjusting the parameter of the phrap. By counting the number of ESTs in each contig, we displayed gene expression patterns in their tissues. We called this the “Virtual Display: VD”. VD allows us to trace the global gene expression profiles of interest during the wheat life cycle, and is going to be opened. Then we collected further RNAs from the stressed

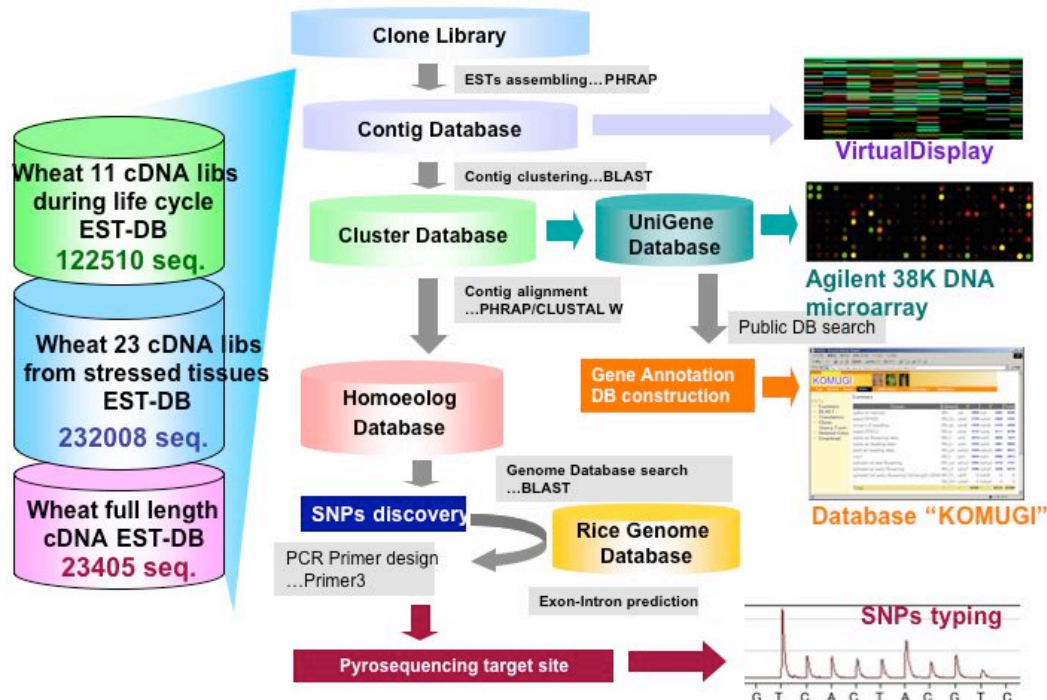


Figure 1. Wheat ESTs Clustering Pipeline : WCEP.

tissues such as drought, salt, cold/heat shock, cultures, metals, and disease fungi as well as additional tissues during the life cycle. In total, gene expression patterns from the 56 tissues and/or stress-treatments, at present can be displayed. More than 630 thousands wheat EST data are available, and these ESTs were classified into ca. 38 thousands gene clusters. Since total gene numbers were predicted to be ca. 40 thousands from the entire sequencing of rice genome, more than 90 % of genes can be captured even in wheat. By applying these cDNA sequence data, we constructed the agilent oligo DNA microarray harboring 38K gene probes. The wheat oligo DNA microarray is now available from our lab (yogihara@yokohama-cu.ac.jp). In addition to ordinary cDNA libraries of common wheat, we had constructed the wheat full length cDNA library. We extracted total RNAs from 13 tissues and/or stress-treatments. These RNAs were mixed, and

supplied for construction of the full length cDNA library with the CAP-trapper method. Approximately 20,000 clones were picked up to carry out one pass sequencing from both ends of inserts. By grouping these cDNA sequences, 6,162 clones were selected to complete the entire sequencing of inserts.

Furthermore, we had constructed the genomic library of Chinese Spring wheat with the transformation-competent artificial chromosome (TAC vector). This TAC library covers ca. three times of the Chinese Spring wheat genome to be proven the selection of single copy gene from the library. In response to user's request, we selected positive clones against the special targets.

These lines of activities indicate that DNA resources of common wheat are substantial and useful, and are required to complete their line-ups in the next stage (Fig. 1).



## Meeting Reports

# The Triticeae Meeting of Japan, 2007

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The Second Triticeae Meeting of Japan was held at WeNARC, Fukuyama on November 17 and 18, 2007. Eighty-two researchers including students from universities and institutes participated in the meeting (Fig. 1). We had twelve oral and 40 poster presentations. The abstracts and poster titles are listed below. The following fields relating Triticeae were presented; molecular biology, genomics, molecular cytogenetics, physiology, genecology, evolution, cereal science, breeding and activities to promote the local wheat production for local consumption. Young researchers had a good opportunity to know a wide field of research. The meeting was a great success with active and fruitful discussion. Next meeting will be held at Okayama University in December 2008. We thanked participants to join the meeting.

## ABSTRACTS & TITLES

### Oral Presentation

#### **01. Analysis of gluten proteins for improvement of Japanese wheat quality**

##### **Kanenori Takata**

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Wheat breeding for bread-making has been conducted actively from 1990s. Ten wheat varieties for bread released for the last decade in Japan. We studied relationships between high molecular weight glutenin subunits (HMW-GS) and bread-making quality. Subunits 5+10 encoded by *Glu-D1d* was associated with a good bread-making quality. Subunits 2.2+12 encoded by *Glu-D1f*, which were found in many Japanese wheat varieties, was associated with a weak dough property. We revealed that the effects of combinations among *Glu-1* loci on physical dough

property using near isogenic lines. Two combinations of *Glu-A1c* / *Glu-D1f* and *Glu-B1e* / *Glu-B1f* especially showed large negative effects on dough strength. We also studied low molecular weight glutenin subunits (LMW-GS) by two-dimensional polyacrylamide gel electrophoresis, which is a powerful tool to decide of LMW-GS genotypes. Subunits encoded by *Glu-B3g* were associated with overly strong dough property on bread-making. Moreover we found that the subunits encoded by *Glu-B3g* improved white salty noodle quality, especially a handling during noodle making. Although gliadins have been disregarded in our breeding program, we have recently found that the quantity of gliadins also affect on physical dough property. To improve the dough property, we need to consider the effects of these gluten proteins and interactions among them. We have developed PCR-makers to select them to accelerate our breeding programs. The varieties with better gluten properties are expected to be released in the next decade.

#### **02. Improvement of wheat transformation and use for functional genomics**

##### **Taiichi Ogawa, Hiroyuki Kawahigashi and Hirokazu Handa**

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Isolation of agriculturally important genes has been accelerated in wheat by development of comparative genomics. Genetic transformation of wheat is considered to be a necessary tool for the determination of gene functions. However, the ability to transform wheat is currently restricted to a few laboratories in Japan. We directly introduced the protocol of wheat transformation using a particle bombardment method from International Maize and

Wheat Improvement Center. In this seminar, we presented the outline of this protocol and discussed factors affecting transformation efficiencies. We produced the transformed wheats constitutively expressing *WFT* gene, which is involved in flowering process, by the particle bombardment method. Using *WFT* transformants, we have obtained valuable information to understand gene network involved in flowering process in wheat. In order to improve transformation efficiency, we established a transformation method using a mutated rice acetolactate synthase gene as a new selection marker. We presented this new selection system for wheat transformation in this seminar.

### **O3. Identification of tolerant genes related to mineral stress in barley**

**Jian Feng Ma**

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Barley is cultivated widely in the world and has developed strategies to overcome various stresses. In terms of mineral stress, barley is characterized by high tolerance to Fe deficiency and low tolerance to Al toxicity. Barley secretes phytosiderophores (mugineic acids) from the roots in response to Fe-deficiency and then takes up Fe in the form of Fe(III)-phytosiderophore complex. We have cloned a gene encoding a transporter (*HvYS1*) for this complex. *HvYS1* gene was mainly expressed in the roots and the expression was enhanced under Fe-deficiency. In situ hybridization and immunostaining revealed that *HvYS1* was localized at epidermal cells of roots. Furthermore, *HvYS1* showed strict specificity for both metals and ligands.

On the other hand, although barley is an Al-sensitive species, there is a large genotypic variation in Al tolerance between cultivars. Secretion of citrate has been associated with Al tolerance in barley. We identified a gene (*HvAACT1*) responsible for the Al-activated citrate secretion by fine mapping combined with microarray analysis, using Al-tolerant cultivar, Murasakimochi and Al-sensitive cultivar, Morex. This gene was constitutively expressed mainly in the roots of Al tolerant barley cultivar. Heterologous expression of *HvAACT1* in *Xenopus* oocytes showed efflux activity for citrate, but not for malate. Over-expression of this gene in tobacco enhanced citrate secretion and Al resistance compared to the wild type plants. *HvAACT1* was localized at the plasma membrane of the epidermal cells in the barley root tips. A good correlation was found between the expression of *HvAACT1* and citrate secretion in 10 barley cultivars differing in Al resistance, suggesting that high expression of this gene is required for Al tolerance.

### **O4. Production and characterization of TILLING lines in Chinese Spring wheat**

**Takehiro Imai<sup>1</sup>, Kanako Kawaura<sup>1</sup>, Masayuki Isshiki<sup>1</sup>, Keiichi Mochida<sup>2</sup>, Shuhei Nasuda<sup>3</sup>, Kazuo Shinozaki<sup>2</sup> and Yasunari Ogihara<sup>1</sup>**

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<sup>2</sup>: RIKEN Plant Science Center

<sup>3</sup>: Graduate School of Agriculture, Kyoto University

TILLING (Targeting Induced Local Lesions In Genomes) is conveniently used for reverse genetic method. Mutated lines were usually produced with the chemical mutagenesis such as EMS, and mutated points were possibly detected by the PCR-based screening. In order to carry out reverse genetics in hexaploid wheat, we tried to produce TILLING lines of Chinese Spring wheat by the EMS treatment.

Seeds were treated with EMS, grown as M<sub>1</sub> plants and self-pollinate to establish M<sub>2</sub> lines. M<sub>2</sub> seeds (3307 lines) were sowed and self-pollinated (as M<sub>3</sub> seeds). Out of 2430 M<sub>2</sub> lines sowed in the last season, 2243 plants were grown in the field (92%) to check their phenotypes. Approximately 10% of M<sub>2</sub> plants showed mutated phenotypes. The observed phenotypes were speltoid and compactoid phenotypes of spike, awn in spike, bush, abnormal phenotype, decreasing number of tillers in plant form, striped, variegated or rolled leaves, and lesion mimic. These observations suggested that the TILLING lines harbored a number of mutations in wheat genes. To estimate mutation rate in the wheat TILLING lines, we investigated the *waxy* locus in the D genome. We screened the mutated genes in *Wx-D1* locus located on the 7D from the pooled M<sub>2</sub> lines with the SURVEYOR method. We identified 10 mutated lines in 444 lines examined, suggesting that total TILLING lines include approximately 80 mutated lines in the *Wx-D1* region. TILLING lines were estimated to contain a mutation per every 40kbp in the D genome. We are going to test mutation rates for other genomes and other genes.

### **O5. Cultivation and utilization of *Triticum aestivum* ssp. *spelta* (L.) Thell. in central Europe**

**Naoki Mori<sup>1</sup> and Shoji Ohta<sup>2</sup>**

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Common wheat (*Triticum aestivum* L., 2n=6x=42) is classified into six subspecies, *i.e.*, ssp. *aestivum* (L.) Thell., ssp. *compactum* (Host.) Mac Key, ssp.

*sphaerococcum* (Perc.) Mac Key, ssp. *macha* (Dekapr. & Menabde) Mac Key, ssp. *spelta* (L.) Thell. and ssp. *vavilovii* (Jakubz.) A. Love, all of which are cultivated forms (Mac Key 1966). Among them, ssp. *aestivum* represents the most common and widely cultivated bread wheat. Two subspecies *compactum* and *sphaerococcum* are other free-threshing forms (non-hulled or naked) of common wheat. Three subspecies *macha*, *spelta* and *vavilovii*, are all non-free-threshing (hulled) and thus seemingly primitive types of common wheat. Current cultivation of ssp. *spelta* can be seen locally in central Europe, northern Spain and western Iran, while ssp. *macha* is endemic in Transcaucasia. In 1997 we participated in the field expedition conducted by Gifu University (The Gifu University Science Exploration in the Mediterranean Region in 1997, GSEM97), and visited central Europe for studying the cultivation and utilization of hulled wheat. In the present meeting, an endemic cultivation and a traditional usage (production of Grünkern) of ssp. *spelta* in southern Germany was reported. In addition, the cultivation of ssp. *spelta* in Switzerland and Austria was also briefly reported.

#### **06. Homoeologous gene-specific regulation of MADS-box genes in polyploid wheat**

**Naoki Shitsukawa and Koji Murai**

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Flower development has been the subject of intensive study over the last decade, particularly in two dicot species, *Arabidopsis* and *Antirrhinum* and these studies led to the description of the ABCDE model. All genes involved in this model, except for *APETALA2*, belong to MADS-box gene family that encode MADS-box transcription factor. A recent study of the ABCDE genes in monocot species, such as rice (*Oryza sativa*), suggests that this model could essentially be extended to monocots, except for the role of the class A genes. Bread wheat is a hexaploid species with A, B, and D genomes derived from the ancestral diploid species. Accordingly, floral organ MADS-box genes may present as triplicated homoeologous genes (homoeologs). To clarify the relevance of homoeologs in flower formation, we analyzed gene structure, expression patterns, and protein functions in the wheat MADS-box genes.

There are two class E genes, *wheat SEPALLATA (WSEP)* and *wheat Leafy Hull Sterile1 (WLHS1)*. The homoeologs of *WSEP* showed similar genomic structures and expression profiles. By contrast, the three homoeologs of *WLHS1* showed genetic and epigenetic alterations. The A genome *WLHS1* homoeolog (*WLHS1-A*) had a structural alteration that contained a large novel sequence of the K domain

sequence. By comparing the structure of *WLHS1-A* locus in diploid, tetraploid, and hexaploid species of *Triticum*, only tetraploid wheat *T. dicoccum* and some of the hexaploid wheat carried the variant *WLHS1-A*. These findings indicate that the sequence change in *WLHS1-A* occurred in a lineage of *T. dicoccum* and that hexaploid species originated on multiple occasions from the domesticated tetraploid species and *Ae. tauschii*. Furthermore, the B genome homoeolog, *WLHS1-B* was predominantly silenced by cytosine methylation. Epigenetic silencing in *WLHS1-B* is reversible regulation, thus using *WLHS1-B* as a model system we will gain further insight the molecular mechanism of the effect of interaction between homoeologous genes during allopolyploidization.

#### **07. Structure of centromeric regions of barley as revealed by molecular cytogenetic analyses**

**Shuhei Nasuda**

Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University

A decade has passed since scientists started to characterize in detail the structures of plant centromeres. Plant centromeres, as well as animal centromeres, are large in size and complex in their constitutions. Although the functions of centromeres are conserved, their nucleotide sequences are diverged. The structural similarity among centromeres from different taxonomic groups is that the centromeric regions are rich in satellite repeats and centromere-specific retrotransposons. Barley centromeres also possess a satellite repeat '(AGGGAG)<sub>n</sub>' and a centromeric Ty3/gypsy retrotransposon '*cereba*'. Our chromatin immunoprecipitation experiments showed that CENH3 protein interact with both (AGGGAG)<sub>n</sub> satellite and *cereba*. There was no significant difference between affinity of CENH3 to (AGGGAG)<sub>n</sub> satellite and that to *cereba*. These results indicated both (AGGGAG)<sub>n</sub> satellite and *cereba* are key sequences that form normal barley centromeres.

Barley chromosomes introduced to wheat background is a good material to study barley centromere structures, because barley chromosome is dispensable and could be modified by biotic and abiotic methods. We found barley telosomes 7HS\* and 7HS\*\* that does not have centromeric repetitive sequences. These chromosomes are transmitted normally in mitosis and meiosis, indicating the presence of fully functional centromeres. The origin of the centromeres on the 7HS\* and 7HS\*\* chromosomes remains to be elucidated. First, the hypothesis that normal 7H chromosome has kinetochore in the pericentromeric region of its short arm was ruled out by direct visualization of the position of spindle-attachment. Fine deletion mapping of barley ESTs revealed that telosomes 7HS\* and

7HS\*\* were derived from multiple rearrangements. The 7HS\* and 7HS\*\* chromosomes retains an EST sequence whose homologue can be found in functional centromere on rice chromosome 8. We are currently testing the 'epigenetic modification' hypothesis that centromeres of 7HS\* and 7HS\*\* have been established through expansion of centromeric chromatin from the chromosome region carrying the EST sequence to its vicinities.

#### **O8. Functional analysis of a barley specific protein P23k by virus-induced gene silencing**

##### **Shin-ichiro Kidou**

Cryobiosystem Research Center, Faculty of Agriculture, Iwate University, Morioka, Iwate, 020-8550 Japan

P23k is a monocot-unique protein that is highly expressed in the scutellum of germinating barley seed. Our expression analyses in barley seed have suggested that P23k is involved in sugar translocation and/or sugar metabolism. However, the role of P23k in barley physiology remains unclear. To gain a better understanding of P23k, a loss of function analysis of P23k in barley is required. Virus-induced gene silencing (VIGS) has recently been developed as an mRNA suppression technique to characterize the function of plant genes, and some silencing of pathogen-related genes were reported in barley and wheat. In the present study, we used *Barley striped mosaic virus* (BSMV)-based VIGS technique to analyze the function of P23k. VIGS of the P23k gene led to abnormal leaf development such as asymmetric orientation of main veins and cracked leaf edges caused by mechanical weakness. Expression analysis of P23k mRNA showed the localization of their transcripts to the vascular bundles and sclerenchyma, where secondary wall formation is most active. In addition, histochemical analyses indicated that the distribution of P23k in leaves coincides with the distribution of cell wall polysaccharides. Considering these results together, it is proposed that P23k is involved in the synthesis of cell wall polysaccharides and contributes to secondary wall formation in barley leaves.

#### **O9. Genetics of microstructure of wheat seed and food products. - DNA tells everything? -**

##### **Tatsuya M. Ikeda**

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In contrast to a recent drastic increase of gene

sequence information in wheat and barley, our knowledge of their various phenotypes has not expanded as well. For example, wheat breeders have been seeking to improve milling efficiency, however the milling efficiency is a complex characteristics related to the amounts of large and small bran consisting of outer layers, aleurone layer and endosperm. Few studies revealed the genetic control of factors involved in these characters. Based on a study on microstructure of wheat seed sections by a scanning electron microscopy and fluorescence microscopy, we found variations on the thickness of the outer layer and cell walls in the endosperm among cultivars. Cultivars showing good milling efficiency had thinner outer layers and very thin cell walls in the central part of the endosperm. These characters seem to be controlled by genes related to cell wall synthesis. To understand relationships of genes with various phenotypes of wheat and barley, we also need to study these phenotypes in microstructural level.

#### **O10. The analysis of proteins related to beer brewing**

##### **Takashi Imure**

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Proteins have important roles in each brewing process. However, there is little information on the relationship between individual protein species and beer quality except for a few findings such as beer foam stability and haze formation. In this study, we analyzed and identified beer proteins using two-dimensional gel electrophoresis (2DE) and mass spectrometry. To identify protein species, we used both disclosed databases such as NCBI-nr and HarVEST unigene, and novel databases containing barley cDNA and EST sequences constructed by Okayama University. As a result, we constructed a novel beer proteome database containing 22 protein species from barley and 2 protein species from yeast. Using this proteome database, we compared the 2DE patterns of the beer proteins among 7 malt cultivars. In consequence, we revealed that spot intensity of several proteins were different between malt cultivars. Moreover, we analyzed beer and haze proteins using 2DE. As a result, we identified barley dimeric alpha-amylase inhibitor (BDAI-I) as one of foam-promoting proteins, and BDAI-I and CMb component of tetrameric alpha- amylase inhibitor (CMb) as one of haze-active proteins. It is suggested that we could apply these results to barley breeding and process control of beer brewing to optimize/improve beer foam stability and haze formation.

## **O11. Genetic improvement for seed composition and quality in hull-less barley breeding**

### **Takashi Yanagisawa**

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Hull-less barley has been used for foods such as miso and boiled pearled barley in Japan. Breeding efforts for genetic improvement for seed composition and quality have done in hull-less barley. The high whiteness of pearled grain is important purpose in our breeding program.

Modern and agriculturally improved hull-less waxy barley cultivar "Daishimochi," was developed using indigenous hull-less waxy barley. The flour of "Daishimochi" and wheat flour mixtures are used for making bread, biscuits, cake, and noodles. In contrast to other cereals, indigenous waxy barley lines have storage starch containing 2-10% amylose. Amylose-free hull-less waxy mutant was induced by chemical mutagen and agriculturally improved high yielding line was recently tested in the performance test for recommended variety. The waxy phenotype produces a stickiness of texture most of Japanese prefer, so the potential uses of waxy barley is expected to expand.

Barley is susceptible to a browning reaction after heating and browning reaction is correlated to its polyphenol content. Proanthocyanidin is a kind of polyphenol, so proanthocyanidin-free mutants were useful to reduce the browning reaction in boiled pearled barley. Proanthocyanidin-free (*ant13*, *ant28*) hull-less barley were recently tested in the performance test for recommended variety.

(1,3) (1,4)- $\beta$ -D-glucan (beta-glucan) is major components of polysaccharides in cell walls of barley endosperm. Beta-glucan is dietary fiber, is favorable for human foods because it lowers cholesterol. Waxy barley has higher concentration of beta-glucan, but takes longer pearling time than non-waxy barley. Beta-glucan content is correlated to the hardness of kernel in barley.

Recently whole barley grain barley and certain dry milled barley grain products are appropriate sources of beta-glucan soluble fiber for the health claim in USA, so barley has the possibility of the functional foods. The uses of improved barley varieties for seed composition and quality will be expanded.

## **O12. The action from growing wheat to baking bread by citizen, which grow their love toward their local cultivar**

### **Tadashi Takahashi**

Faculty of Agriculture, Yamaguchi University

Yamaguchi Prefecture's government drives its citizen to a consumer behavior "Chisan Chisho." The Chisan Chisho means that the citizen consumes the local products produced in the neighborhood. The wheat grains harvested in the neighborhood are milled into flour at the neighbor factory, and then the flour is made into bread at the neighbor bakery. That is the Chisan Chisho for wheat. At 2003 the government selected a wheat cultivar "Nishinokaori" for Chisan Chisho bread in Yamaguchi. Then it has made brochures, has carried out some projects and has taken some presentations to have its citizen know the "Nishinokaori". At the same time, the citizen also opened the Wheat-Bread Associates to take action for Chisan Chisho by themselves. A member of the associates talks about wheat and bread to each other through E-mail. In addition they grow wheat in the local field at Mistuo Shunan. They also mill their own flour from their wheat product and bake bread from their flour. The Yamaguchi citizen has been getting to love "Nishinokaori" as their own cultivar throughout the government's and the citizen's action. Now, the associate member is planning to have the wheat farmers sell their flour to the Yamaguchi citizen. The flour is packed into small bag, 200g for one time use, by farmers. They expect more citizen to love Nishinokaori through making bread from the flour by themselves.

### **Poster Presentation**

- P1.** Kouyama, S., K. Kawaura, Y. Ogihara (Kihara Inst. Biol. Res., Yokohama City U.) Analyses of global gene expression patterns in wheat through the allopolyploidization.
- P2.** Mizuno, N., S. Takumi (Lab. Plant Genet., Grad. Sch. Agr. Sci., Kobe Univ.) cDNA-AFLP analysis of seedling leaves in synthetic hexaploid wheat.
- P3.** Yasumoro, M., K. Kawaura, Y. Ogihara (Kihara Inst. Biol. Res., Yokohama City U.) Molecular study on regulation systems of gene expression in hexaploid wheat.
- P4.** Siniauskaya, M. G., C. Nakamura (Lab. Plant Genet., Grad. Sch. Agr. Sci., Kobe Univ.) The development of chloroplast macroarray system in wheat.
- P5.** Nankaku, N.<sup>1</sup>, T. Iimure<sup>2</sup>, K. Sato<sup>1</sup> (1. Research Institute for Bioresources, Okayama Univ., 2. Sapporo Brewery Ltd.) Establishment of proteomics analysis system in barley.
- P6.** Takaku, M., T. Imai, K. Kawaura, M. Isshiki, Y. Ogihara (Kihara Inst. Biol. Res., Yokohama City U.) Variation of the spike morphology in TILLING lines of common wheat.
- P7.** Kawaura, K., M. Isshiki, Y. Ogihara (Kihara Inst. Biol. Res., Yokohama City U.) Expression analysis of *APETALA2* (*AP2*)-like genes in

- hexaploid wheat.
- P8.** Kitagawa, S., K. Murai (Dept. Biosci., Fukui Pref. Univ.) Comparative analysis of two *CONSTANS*-like genes in wheat.
- P9.** Zhu, Y., K. Murai (Dept. Biosci., Fukui Pref. Univ.) *ORF260*, a candidate of pistillody-related mitochondrial gene in alloplasmic wheat.
- P10.** Kinjyo, H., K. Murai (Dept. Biosci., Fukui Pref. Univ.) Comparative analysis of three *APETALAI/FLUITFULL*-like genes in wheat.
- P11.** Suzuki, T., K. Murai (Dept. Biosci., Fukui Pref. Univ.) A triangle model of WAP1-WFT-VRN2 for flowering in wheat.
- P12.** Nishizawa, S., K. Murai (Dept. Biosci., Fukui Pref. Univ.) Identification of wheat homolog of *UNUSUAL FLORAL ORGAN (UFO)*-like gene.
- P13.** Hatano, H., S. Takumi (Lab. Plant Genet., Grad. Sch. Agr. Sci., Kobe Univ.) cDNA-AFLP analysis of pistil-like structures in alloplasmic wheat.
- P14.** Taniguchi, K., A. Horikawa, T. Terachi (Fac. Eng., Kyoto Sangyo U.) Proteomic analysis of alloplasmic wheat with *Ae. mutica* cytoplasm.
- P15.** Nakanishi K., K. Taniguchi, A. Horikawa, T. Terachi (Fac. Eng., Kyoto Sangyo U.) Analysis of specific mitochondrial genes on alloplasmic wheat with *Ae. mutica* cytoplasm.
- P16.** Kishii, M.<sup>1</sup>, T. Ban<sup>1,2</sup>, G.V. Subbarao<sup>1</sup>, H. Tsujimoto<sup>3</sup>, M. Iwanaga<sup>4</sup> (1. CIMMYT, 2. Kihara Inst. Biol. Res., Yokohama City U., 3. JIRCAS, 4. Fac. Agr., Tottori Univ.) Wheat wild relatives *Leymus racemosus* could boost nitrogen use efficiency of wheat with Biological Nitrification Inhibition (BNI).
- P17.** Mishina, K.<sup>1</sup>, T. Koba<sup>2</sup> (1. Grad. Sch. Sci. Technol., Chiba Univ., 2. Grad. Sch. Horticulture, Chiba Univ.) Observation of pollen tube elongation in relation to the crossability of common wheat with rye.
- P18.** Ueda, T., S. Kikuchi, H. Elamein, H. Tanaka, H. Tsujimoto (Fac. Agr., Tottori U.) The effect of chemical treatments on the chromosome elimination in super-wide hybrids in wheat.
- P19.** Sakuma, S., T. Koba (Lab. Genet. Plant Breed., Grad. Sch. Horticulture, Chiba Univ.) Variation in crossability of tetraploid wheat with rye.
- P20.** Nishinaka, M.<sup>1</sup>, M. Kato<sup>1</sup>, Y. Okumoto<sup>1</sup>, K. Kato<sup>2</sup>, T. Tanisaka<sup>1</sup> (1. Lab. Plant Breed., Grad. Sch. Agr., Kyoto Univ., 2. Fac. Agr., Okayama Univ.) Diversity and distribution of High-Molecular-Weight glutenin subunits in Asian wheat.
- P21.** Terasawa, Y.<sup>1</sup>, K. Takata<sup>2</sup>, T. Ban<sup>1</sup>, T. Sasanuma<sup>1</sup> (1. Kihara Inst. Biol. Res., Yokohama City U., 2. Natl. Agr. Res. Cent. Western Region) Genetic diversity of wheat landraces in Tibet.
- P22.** Kato, M.<sup>1</sup>, Y. Okumoto<sup>1</sup>, M. Nishinaka<sup>1</sup>, K. Kato<sup>2</sup> (1. Lab. Plant Breed., Grad. Sch. Agr., Kyoto Univ., 2. Fac. Agr., Okayama Univ.) The diversity of the Low Molecular Weight Glutenin Subunits in Asian wheat and their effects on SDS sedimentation volumes.
- P23.** Ohmichi, Y., N. Mori (Lab. Plant Genet., Grad. Sch. Agr. Sci., Kobe Univ.) Molecular variation in mitochondrial DNA SSRs in ancestral species of wheat.
- P24.** Takumi, S. (Lab. Plant Genet., Grad. Sch. Agr. Sci., Kobe Univ.) Identification of hexaploid wheat accessions with a null allele of *Wknx1b*: Implication to speciation of *Triticum carthlicum*.
- P25.** Manickavelu, A., K. Kawaura, H. Imamura, M. Mori, Y. Ogihara (Kihara Inst. Biol. Res., Yokohama City U.) Construction of genetic linkage map for Chinese Spring x Spelta population of common wheat using SSR markers.
- P26.** Fujita, Y.<sup>1</sup>, H. Fukuoka<sup>2</sup>, H. Yano<sup>1</sup> (1. Natl. Agr. Res. Cent. Western Region, 2. NIVTS) Identification of wheat cultivars in wheat food products using SSR markers.
- P27.** Manangkil, O.E.<sup>1</sup>, N. Mori<sup>1</sup>, H. T. T. Vu<sup>1</sup>, T. Ishii<sup>2</sup>, S. Yoshida<sup>1</sup>, C. Nakamura<sup>1</sup> (1. Lab. Plant Genet., Grad. Sch. Agr. Sci., Kobe Univ., 2. Lab. Plant Breed., Grad. Sch. Agr. Sci., Kobe Univ., 3. Hyogo Pref. Inst. Agr.) QTLs controlling seedling-vigor at germination stage under submergence in rice.
- P28.** Hoshikawa, A., K. Kawaura, Y. Ogihara (Kihara Inst. Biol. Res., Yokohama City U.) Functional analysis of wheat transcription factors in response to salt stress.
- P29.** Terashima, A., S. Takumi (Lab. Plant Genet., Grad. Sch. Agr. Sci., Kobe Univ.) Nucleotide diversity of drought-responsive genes in *Aegilops tauschii*.
- P30.** Hatta, K., S. Oda, M. Fujita (Natl. Agr. Res. Cent. Kyushu and Okinawa region) Screening of the resistant wheat varieties (*Triticum aestivum* L.) to Wheat Yellow Mosaic Virus (WYMV) isolate newly found at Kyushu region in Japan.
- P31.** Kubo, K., N. Kawada, K. Hatta, M. Fujita, S. Oda (Natl. Agr. Res. Cent. Kyushu and Okinawa region) Evaluation of resistance to spread of Fusarium head blight in wheat by degree of rachilla and rachis browning.
- P32.** Mizukami, M., K. Murai (Dept. Biosci., Fukui Pref. Univ.) Genetic and molecular mechanism of heading in extra early-heading wheat varieties.
- P33.** Okumura, Y., S. Takumi (Lab. Plant Genet., Grad. Sch. Agr. Sci., Kobe Univ.) Nucleotide diversity of photoperiod sensitivity-related genes in *Aegilops tauschii*.
- P34.** Garg, M.<sup>1</sup>, H. Tanaka<sup>1</sup>, N. Ishikawa<sup>2</sup>, K. Takata<sup>2</sup>, M. Yanaka<sup>2</sup>, H. Tsujimoto<sup>1</sup> (1. Fac. Agr., Tottori U., 2. Natl. Agr. Res. Cent. Western Region) Seed storage proteins in Triticeae: a novel source of variation for improvement of wheat flour quality.
- P35.** Saito, M., M. Ishiki, K. Kawaura, Y. Ogihara (Kihara Inst. Biol. Res., Yokohama City U.) Molecular analyses of seed storage proteins in

bread wheat.

**P36.** Araki, E.<sup>1</sup>, T. M. Ikeda<sup>1</sup>, Y. Ogihara<sup>2</sup>, A. Toyoda<sup>3</sup>, H. Yano<sup>1</sup> (1. Natl. Agr. Res. Cent. Western Region, 2. Kihara Inst. Biol. Res., Yokohama City U., 3. RIKEN) Development of transgenic rice expressing wheat high- and low-molecular-weight glutenin subunit proteins.

**P37.** Tanaka, H., H. Tsujimoto (Fac. Agr., Tottori U.) Group-1 chromosome deletions affect on dough strength in common wheat.

**P38.** Yanaka, M., K. Takata, N. Ishikawa, T. M. Ikeda (Natl. Agr. Res. Cent. Western Region) Relationship between endosperm cell wall thickness and milling efficiency in wheat.

**P39.** Tonooka, T., E. Aoki, T. Yoshioka (Natl. Inst.

Crop Sci., NARO) Development of proanthocyanidin-free NILs in barley.

**P40.** Takahashi, A., T. M. Ikeda, T. Takayama, T. Yanagisawa (Natl. Agr. Res. Cent. Western Region) Analysis of grain-hardness related hordoindolines in barley. -Grain-hardness of in segregating population with the differences of the number of HINB-



**Figure 1.** The second Triticeae Meeting of Japan – Participant Group Photo.





## Others

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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.

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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](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).

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

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