



# Wheat Information Service

## Electronic Newsletter for Wheat Researchers

**No. 107**

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## Research Information

# Comparative studies of semi-dwarf wheat genotypes (*Triticum aestivum* L.) for yield and yield components

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## Abstract

In yield comparison line 04 had comparatively the highest grain yield. The subsequent lines 01, 02, 06, 07 had comparatively higher grain yields. The possible reasons for higher grain yield in line 04 could be due to early heading date, double dwarf plant height and higher number of grains per spikelet and the boldest grain size. Correlation analysis for pooled data was calculated for the genotypes. Plant height had positive and highly significant correlation for spike length, number of spikelets per spike, number of grains per main spike and grain yield of main spike. Grain yield of main spike is a very important character; it has positive and highly significant correlation with all the characters such as plant height, spike length, number of spikelets per spike, number of grains per spike and number of grains per spikelet.

*Key words:* Plant height, semi-dwarfism, wheat genotypes, yield components, agronomic characteristics, correlation studies

## Introduction

The control of plant height in cereals is known to be complex because of its polygenic nature and subject to environmental effects. Tall wheat cultivars and lines (*Triticum aestivum* L.  $2n=6x=42$ ) are more prone to lodging, particularly when in favourable environments whereas semi-dwarf cultivars are shorter less prone to lodging (Ahmad and Sorrells 2002). The primary sources of semi-dwarfism in wheat are the Norin 10 reduced height genes, *Rht1* and *Rht2*. These genes are located on the 4B and 4D chromosomes of wheat (Gale et al. 1975; Gale and Marshall 1973). Sayre et al. (1997) suggested the strong relationship between grain yield and harvest index and grain yield and kernels per square meter. Jamali et al. (2003) reported that the important character plot grain yield was not correlated with days to heading, plant height, number of spikelets, number of grains per spike, main spike grain yield, grain weight, number of grains per spikelet, plot grain yield and harvest index. The aim of present study was to compare the genotypes for yield and yield components and their relationship with plant height.

## Materials and methods

Sixteen advance entries were selected and evaluated for yield and yield components with two check varieties viz. Sarsabz and Kiran-95. The genotypes were planted into four rows each with row length of 03 meters. The experiment was laid out in a Randomized Complete Block Design with three replicates. Weekly mean temperature during the crop ranged from 12.2 °C (minimum) to 28.84 °C (maximum) and average humidity remained at 71.56. Five plants from each replicate were selected at random for recording the data. The statistical data analyzed according to Steel and Torrie (1981).

## Results and discussion

### Comparative performance

The results of yield and yield components are presented in Table 1. The yield comparison studies showed that line 04 had comparatively the highest grain yield. The subsequent lines which had higher grain yields were lines 01, 02, 06, 07.

**Table 1.** Comparative studies of wheat genotypes for yield and yield components

No.	Genotypes	Days to heading	Plant height (cm)	Spike length (cm)	Spikelets per spike	No. of grains per spike	Grain yield of spike (g)	Grains per spikelet	Plot yield (g)	1000-Grain weight (g)
1	C6-98-03	71g	53.4i	9.75g	16ghi	48.6cdef	1.82bc	3.02ab	625ab	38.11ab
2	C6-98-04	71g	54.9i	8.05h	14j	40.4gh	1.48e	2.48abcde	588abc	36.94bc
3	C6-98-05	71g	86ab	10.1fg	16.5fgh	49.9bcdef	1.94b	3.01ab	563abcd	39.16ab
4	C6-98-06	72g	61.2g	9.85fg	15.1hij	44.6efgh	1.51e	2.93abcd	675a	40a
5	C6-98-11	85bc	63fg	10.45efg	17efg	39.9h	1.54de	2.35gh	300f	38.77ab
6	C6-98-12	73fg	61.8g	10.45efg	16.9efg	51.4bcde	2.02a	3.02ab	575abc	37.89ab
7	C6-98-13	80cde	64.2f	10.65def	17.7def	48def	1.87bc	2.7cdef	575abc	38.74ab
8	C7-98-01	84bcd	45.6j	10.3fg	18.1cde	44fgh	1.5e	2.48fg	363cdef	34.08de
9	C7-98-02	82cde	57.7h	11.25bcd	18.8cd	48.9cdef	1.67bcde	2.63defg	438bcdef	34.30de
10	C7-98-05	83bcd	84.7b	12.25a	21.2b	55.5abc	1.85bc	2.61efg	463abcdef	33.31def
11	C7-98-08	80cde	67.6e	9.8g	21.6ab	47.1defg	1.64cde	2.17h	538abcde	34.94cd
12	C7-98-10	94a	69de	11.3bcd	21.5ab	55.4abc	1.81bcd	2.59efg	338def	32.57def
13	C7-98-11	78ef	69.9d	11.45bc	19.5c	53.5abcd	1.73bcde	2.74bcdef	463abcdef	32.31ef
14	C7-98-12	87b	66.9e	11.95ab	22.8a	60a	1.86bc	2.62defg	313ef	31.01f
15	C7-98-13	82bcde	76.8c	10.75cdef	18de	53abcd	1.72bcde	2.95abc	500abcdef	32.54def
16	C7-98-15	82bcde	78.8c	11.2bcde	21b	52.8abcd	1.7bcde	2.51f	375cdef	32.14ef
17	Sarsabz	73fg	86.6ab	10fg	14.9ij	44fgh	1.5e	3.02ab	450abcdef	34.34de
18	Kiran-95	79de	88.1a	12.35a	18.6cd	57ab	1.85bc	3.06a	513bcdef	33.78de

Note: Significance level was at 0.05

The possible reasons for higher grain yield in line 04 could be due to early heading date, double dwarf plant height and higher number of grains per spikelet and the boldest grain size. Villarreal et al. (1992) reported that double dwarf varieties yielded over 2.4% than the single dwarf gene (*Rht2*) varieties. However, the varieties with double dwarf (*Rht1Rht2*) were not significantly different than the varieties carrying *Rht1Rht1*. Higher grain yield in line 01 could be due to its early heading dates, higher number of grains per spikelet and also increased grain weight. Higher grain yield in line 02 may be due to its early heading dates; probably it escapes from the effect of high temperature during grain filling period. The high grain yield in line 06 could be due to early heading dates, the highest main spike grain yield and increased number of grains per spikelet. Waddington et al. (1986), who studied more recent semi-dwarf cultivars pointed out the importance of increased kernels per spike and indicated that the most recent progress (cultivars after 1975) appeared to raise from increased biomass and not increased harvest index. The higher grain yield of line 07 could be due to its bold grains. Shearman et al. (2005) suggested that recent genetic gains in grain yield have been accomplished due to increased number of grains per unit area in modern semi-dwarf wheat varieties.

#### Correlation studies

The correlation studies are presented in Table 2. Combined/ pooled correlation analysis was calculated for genotypes. Plant height has positive and highly significant correlation for spike length, number of spikelets per spike, number of grain and grain yield of main spike. These results suggest that an increase in plant height may increase the spike length, number of spikelets, number of grains per spike and main spike grain yield in semi-dwarf wheat. Li et al. (2006) reported that both the *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) semi-dwarfing genes had significantly positive

effects on kernel number per spike and grain yield per spike. The semi-dwarf genes (*Rht1* or *Rht2*) may increase grain yield, grains per spike, grain weight, grain volume weight, biomass, coleoptile length, stand establishment potential and protein content when compared to their non semi-dwarf alleles (Allan 1983; Gale and Youssefian 1985). Muhammad et al. (2006) reported that plant height had negative correlation with grain yield. However, plant height had non significant correlation with number of grains per spikelets. The results reveal that plant height do not affect the spike fertility. Belay et al. (1993) reported that plant height had a non significant correlation with number of grains per spike and spikelet. Spike length had highly significant positive correlation with number of spikelets per spike, number of grains per spike and grain yield of main spike. These results indicate that an increase in spike length may also increase the number of spikelets per spike, number of grains per spike and grain yield of main spike. However, spike length had negative but non significant correlation with number of grains per spikelet. Number of spikelets per spike had highly significant positive correlation with number of grains per main spike and grain yield of main spike. Jamali et al. (2003) reported that the number of grains per main spike had positive and significant correlation with grain yield of main spike. However, number of spikelets had highly significant negative correlation with number of grains per spikelet. These results suggest that an increase in number of spikelets may reduce the spike fertility. Number of grains per spike had highly significant positive correlation with grain yield of main spike and number of grains per spikelet. Main spike grain yield had highly significant positive correlation with number of grains per spikelet. Grain yield of main spike is a very important character; it has positive and highly significant correlation with all the characters such as plant height, spike length, number of spikelets per spike, number of grains per spike and spikelet. Selection based on grain

**Table 2.** Correlation coefficients for pooled data of yield and yield components.

Characters	Spike length	No. of spikelets	No. of grains per spike	Grain yield of main spike (g)	No. of grains per spikelet
Plant height	0.334***	0.171*	0.254***	0.242***	0.123n.s.
Spike length		0.592***	0.491***	0.357***	-0.07n.s.
No. of spikelets			0.577***	0.299***	-0.366***
No. of grains per spike				0.837***	0.530***
Grain yield of main spike (g)					0.616***

Note: \*=0.05, \*\*=0.01, \*\*\*=0.001 significance level.

yield of main spike could result in better genotypes.

Keeping in view these results, we may conclude that selection based on number of grains per main spike and grain yield of main spike could result in evolution of quality genotypes.

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## Research Information

# Segregation analysis of heading time and its related traits in wheat F<sub>2</sub> populations between two Nepal landraces and a Japanese cultivar Shiroganekomugi

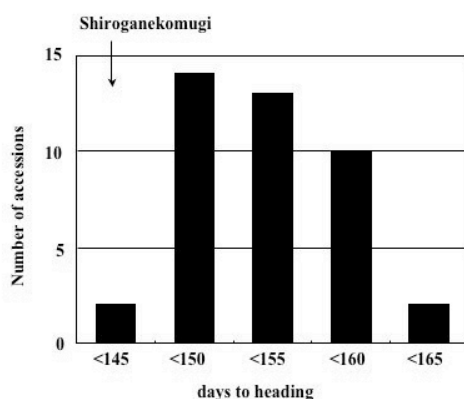
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Heading time is one of the most important traits for wheat improvement. Especially in Japan, rainy season is overlapped with wheat harvesting, resulting in occurrence of pre-harvest sprouting and Fusarium damage, and reduction of grain quality. Genetic control of heading time, therefore, is critical for wheat breeding in Japan. Landraces collected in Nepal, Bhutan and China abundantly provide natural variation (Kihara and Yamashita 1956; Redaelli et al. 1997; Ward et al. 1998).

To study the genetic variation of days to heading, seeds of 41 accessions listed in Table 1 were sown in 19th November 2005 (two seeds per accession) and plants grown under field conditions at Kobe University. Heading time of the landraces and a Japanese cultivar 'Shiroganekomugi' of common wheat (*Triticum aestivum* L.) was scored using the first column of the individual plants. Shiroganekomugi is a standard line for early flowering in Japan. Heading time among the 41 landraces varied from 141 to 160 days (mean =  $153 \pm 2.8$  days) (Table

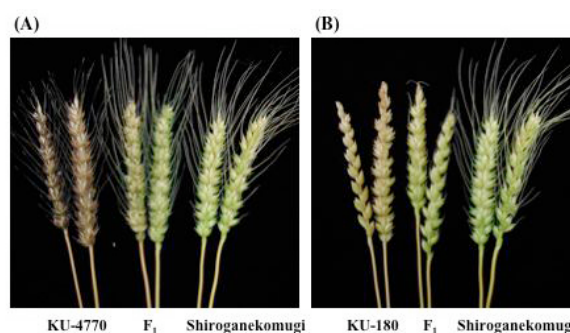


**Fig. 1.** Frequency distribution of days to heading in the examined accessions. An arrow indicates days to heading of Shiroganekomugi.

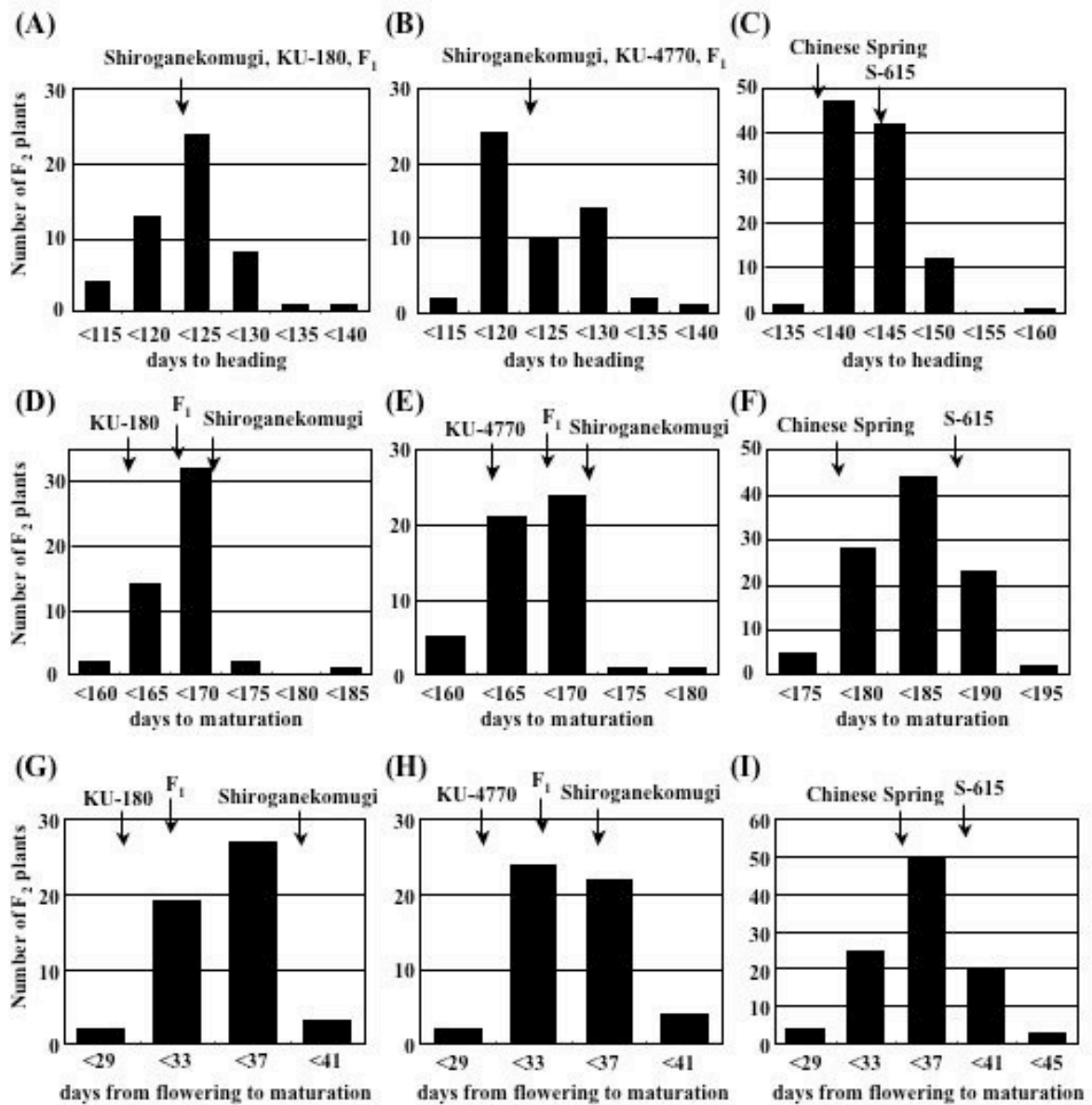
1; Fig. 1). Two Nepal varieties, KU-4770 and KU-180, showed the earliest flowering in the examined accessions.

Next, KU-4770 and KU-180 were crossed to Shiroganekomugi, and heading and flowering time of the F<sub>1</sub> plants was compared with those of the parental lines in 2007-2008. Heading and flowering of the F<sub>1</sub> plants were as early as those of their parental landraces. Spike maturation time was estimated as the date to become completely yellowish peduncle after flowering. The maturation time of KU-4770 and KU-180 were earlier than that of Shiroganekomugi, and days to maturation of the F<sub>1</sub> plants were the middle of the days of parents (Fig. 2).

Seeds of the 51 and 52 F<sub>2</sub> plants, which were respectively selfed progeny of the KU-4770- and KU-180-crossed F<sub>1</sub> plants, were sown in 8th December 2007 and plants grown under field conditions. As a control, F<sub>2</sub> population with 104 plants between common wheat cultivar 'S-615' and 'Chinese Spring' (CS) was additionally used. Three field traits, i.e., heading, flowering and spike maturation dates, were scored in the three F<sub>2</sub> populations. In the F<sub>2</sub>



**Fig. 2.** Spike morphology of F<sub>1</sub> plants between Shiroganekomugi and the Nepal landraces. The spikes were picked up at the same day.



**Fig. 3.** Frequency distribution of flowering-related characters in the three F<sub>2</sub> populations. Arrows indicate the traits of F<sub>1</sub> and their parental accessions.

(A) Days to heading in the Shiroganekomugi x KU-180 F<sub>2</sub> population.

(B) Days to heading in the Shiroganekomugi x KU-4770 F<sub>2</sub> population.

(C) Days to heading in the S-615 x Chinese Spring F<sub>2</sub> population.

(D) Days to maturation in the Shiroganekomugi x KU-180 F<sub>2</sub> population.

(E) Days to maturation in the Shiroganekomugi x KU-4770 F<sub>2</sub> population.

(F) Days to maturation in the S-615 x Chinese Spring F<sub>2</sub> population.

(G) Days from flowering to maturation in the Shiroganekomugi x KU-180 F<sub>2</sub> population.

(H) Days from flowering to maturation in the Shiroganekomugi x KU-4770 F<sub>2</sub> population.

(I) Days from flowering to maturation in the S-615 x Chinese Spring F<sub>2</sub> population.

populations between Shiroganekomugi and Nepal landraces, much earlier- and later-heading plants were transgressively segregated compared with their parental accessions (Fig. 3A, B). On the other hand, heading and flowering dates of most F<sub>2</sub> plants ranged within the dates of their parental lines in the F<sub>2</sub>

population of S-615 and CS (Fig. 3C). Some early- and late-matured F<sub>2</sub> plants were transgressively segregated, but the numbers of transgressive segregants were limited in the three F<sub>2</sub> populations (Fig. 3D-F). Days from flowering to spike maturation of most F<sub>2</sub> plants ranged in the middle of those of their

Table 1. List of wheat accessions used in this study and their days to heading

Accession number	Variety	Country	Days to heading
AS331	ssp. <i>yunnanense</i>	China	160
AS489	cv. Chengdu-Guang-tou	China	151
AS742	cv. Pengan White Wheat	China	151
AS743	cv. Wanxian White Wheat	China	152
AS745	cv. Yongchuan White Wheat	China	158
AS746	cv. Changning White Wheat	China	160
AS750	cv. Rongjing White Wheat	China	154
AS755	cv. Yiling White Wheat	China	147
AS764	cv. Langzhong White Wheat	China	157
AS2228	cv. Chengdu-Guang-tou S-1	China	153
KU-172	<i>turcomanicum</i> Kob.	Nepal	159
KU-174	<i>glaucolutescens</i> Vatz.	Nepal	153
KU-176	<i>albidum</i> Alef.	Nepal	151
KU-178	<i>pseudointermediens</i> Flaksb.	Nepal	152
KU-179	<i>nigricolor</i> Flaksb.	Nepal	146
KU-180	<i>multurum</i> Alef.	Nepal	141
KU-183	<i>caesium</i> Alef.	Nepal	156
KU-4720	<i>subturcicum</i> Vav.	Nepal	155
KU-4735	<i>nigromelanopogon</i> Goekg.	Nepal	156
KU-4736	<i>nigromelanopogon</i> Goekg.	Nepal	152
KU-4737	<i>nigromelanopogon</i> Goekg.	Nepal	153
KU-4755	<i>nigromelanopogon</i> Goekg.	Nepal	156
KU-4758	<i>nigromelanopogon</i> Goekg.	Nepal	153
KU-4761	<i>subnigromelanopogon</i> Goekg.	Nepal	146
KU-4763	<i>subnigromelanopogon</i> Goekg.	Nepal	146
KU-4765	<i>nigromelanopogon</i> Goekg.	Nepal	148
KU-4766	<i>nigromelanopogon</i> Goekg.	Nepal	148
KU-4770	<i>nigromelanopogon</i> Goekg.	Nepal	143
KU-4771	<i>nigromelanopogon</i> Goekg.	Nepal	146
KU-4772	<i>nigromelanopogon</i> Goekg.	Nepal	148
KU-7001	<i>nigromelanopogon</i> Goekg.	Bhutan	148
KU-7002	<i>nigromelanopogon</i> Goekg.	Bhutan	147
KU-7004	<i>albidum</i> Alef.	Bhutan	151
KU-7029	<i>subnigromelanopogon</i> Goekg.	Bhutan	146
KU-7070	<i>nigricolor</i> Flaksb.	Bhutan	151
KU-7073	<i>alborubrum</i> Koern.	Bhutan	156
KU-7074	<i>multurum</i> Alef.	Bhutan	156
KU-7075	<i>albidum</i> Alef.	Bhutan	146
KU-7076	<i>triste</i> Flaksb.	Bhutan	156
KU-7079	<i>nigromelanopogon</i> Goekg.	Bhutan	146
KU-7087	<i>nigromelanopogon</i> Goekg.	Nepal	149
	cv. Shiroganeomugi	Japan	140

KU accessions by Plant Germ-plasm Institute, Kyoto University

AS accessions supplied by Dr. Rick Ward, Michigan State University

parental accessions in the three populations (Fig. 3G-I).

Correlation coefficients among the examined traits were calculated in the three F<sub>2</sub> populations (Fig. 4). In all populations, days to heading, flowering and spike maturation were significantly correlated to each other. Correlations between heading and flowering times and days from flowering to maturation were negative in the F<sub>2</sub> population of Shiroganeomugi and KU-4770, which was not significant in the other populations. Days from flowering to spike maturation were

	days to heading	days to flowering	days to maturation
days to flowering	0.93***		
days to maturation	0.71***	0.75***	
days from flowering to maturation	-0.48***	-0.53***	0.14

(A)

	days to heading	days to flowering	days to maturation
days to flowering	0.96***		
days to maturation	0.72***	0.78***	
days from flowering to maturation	-0.27	-0.23	0.43**

(B)

	days to heading	days to flowering	days to maturation
days to flowering	0.93***		
days to maturation	0.68***	0.71***	
days from flowering to maturation	-0.06	-0.09	0.64***

(C)

Fig. 4. Correlation coefficients ( $r$ ) among the examined traits in the three F<sub>2</sub> populations. \*\*,  $p < 0.01$ , \*\*\*,  $p < 0.001$

(A) Shiroganeomugi x KU-4770 F<sub>2</sub> population.

(B) Shiroganeomugi x KU-180 F<sub>2</sub> population.

(C) S-615 x Chinese Spring F<sub>2</sub> population.

significantly correlated with days to maturation in the F<sub>2</sub> populations between Shiroganeomugi and KU-180 and between S-615 and CS.

These results indicated that genes associated with early-flowering in KU-180 and KU-4770 were different from early-flowering genes in Shiroganeomugi. It is well known that heading time is genetically determined by vernalization requirement, photoperiodic sensitivity and narrow-sense earliness (Yasuda and Shimoyama 1965; Kato and Yamagata 1988). The early-flowering genes in the Nepal landraces might be associated with narrow-sense earliness and useful for breeding of earlier-flowering Japanese cultivars. Genetic mapping of the early-flowering genes in the Nepal landraces should be required for the breeding in the further study. Probably landraces in Nepal and Bhutan provide a lot of agronomically important genes for wheat breeding. Natural variation analyses of these landraces are efficient to identify the useful genes.

#### Acknowledgements

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## Research Information

# Cloning of a wheat cDNA encoding an ortholog of the maize **LIGULELESS4** homeobox protein

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The shoot apical meristem (SAM) plays a central role in plant development, determination of cell fate and differentiation of vegetative tissues. Class I *Knotted1*-like homeobox (*KNOX*) genes maintain the indeterminacy of SAM and act in subsequent shoot development (Kerstetter et al. 1997; Sentoku et al. 1999). A maize semidominant mutation *Liguleless3* (*Lg3*) allele disrupts the leaf at the ligular region of the midrib by transforming blade, auricle and ligule to sheath-like tissue (Fowler and Freeling 1996). The *lg3* gene encodes a homeodomain protein in the *KNOX* family (Muehlbauer et al. 1999). Other dominant leaf mutants such as *Knotted1* (*Kn1*), *Rough sheath1* (*Rs1*) and *Liguleless4* (*Lg4*) exhibit similar blade-to-sheath transformations in maize (Freeling and Hake 1985; Becraft and Freeling 1994; Fowler and Freeling 1996), and the *kn1*, *rs1* and *lg4* genes also belong to the *KNOX* family (Kerstetter et al. 1994; Bauer et al. 2004). Two *KNOX* genes, *Wknox1* and *WRS1*, were isolated in common wheat (Takumi et al. 2000; Morimoto et al. 2005, 2009). The previous expression analyses and transgenic studies indicated that *Wknox1* and *WRS1* are functionally orthologous to *kn1* and *rs1*, respectively. Here, we reported cloning of a cDNA encoding an *lg4* ortholog from young spikes of common wheat.

Partial cDNA sequences of wheat *KNOX* family were previously reported (Takumi et al. 2000). Based on one of the partial sequences, the cDNA-specific primer was designed for 5'- and 3'-RACE PCR to isolate a full-length cDNA sequence. The RACE PCR was performed using mRNA isolated from young spikes of a common wheat (*Triticum aestivum* L.) cultivar 'Chinese Spring' (CS) and SMART RACE cDNA Amplification Kit (Clontech). After sequencing of the RACE PCR products, a full-length cDNA fragment was isolated (Fig. 1).

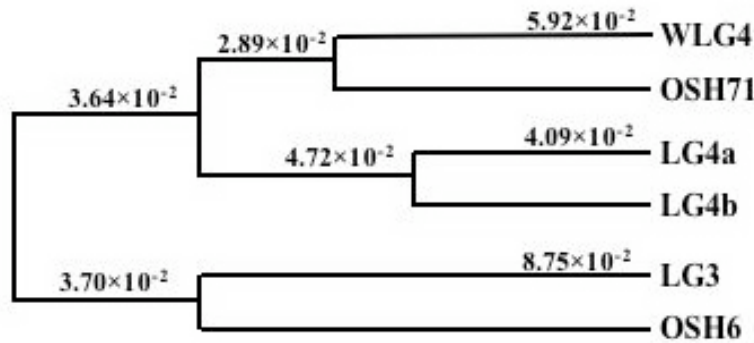
This cDNA contained a complete open reading

frame encoding putatively a KN1-type homeobox protein with 306 amino acid residues that showed amino acid identities of 75.8% with rice OSH71, 73.8% with maize LG4b and 70.9% with maize LG4a (Fig. 2). *lg4a* (formerly *knox11*) and *lg4b* (*knox5*) are likely to encode the redundant function (Bauer et al. 2004), and closely linked duplicates on maize chromosome 8L (Kerstetter et al. 1994). *OSH71* is a rice ortholog of maize *lg4* genes (Sentoku et al. 1999). The high amino acid identities were observed in three conserved domains of the KN1-type homeobox proteins, KNOX, ELK and Homeo domains, among the isolated cDNA-encoding protein, OSH71 and LG4. Our identified cDNA-encoding protein was phylogenetically closer to the LG4 and OSH71 proteins rather than their closely related LG3 and OSH6 (Fig. 3). Therefore, we named the *lg4*-like cDNA as wheat *LIGULELESS4* (*WLG4*). *WLG4* cDNA sequence was deposited in the DDBJ database under the accession number AB465042.

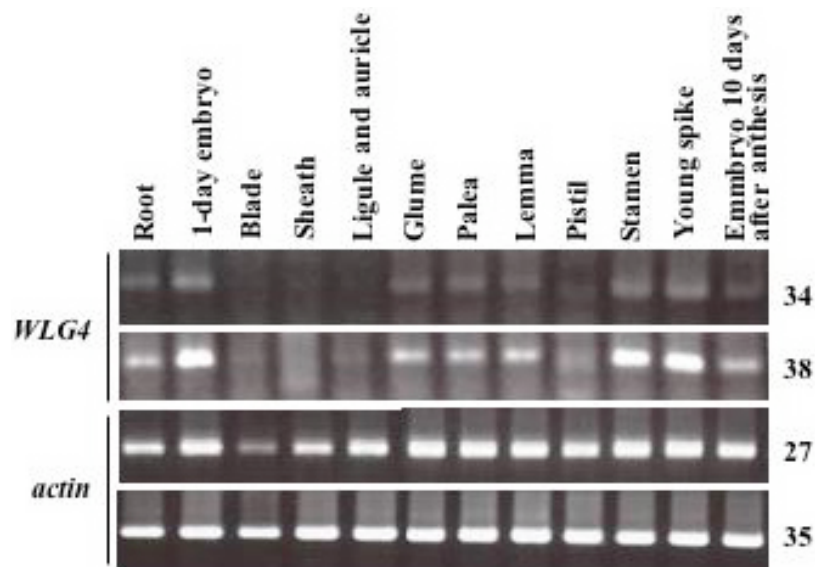
To study the copy number of *WLG4* in the wheat genome, Southern blots were analyzed using total DNA isolated from diploid (*T. monococcum*), tetraploid (*T. durum* cv. 'Langdon') and hexaploid wheat (CS). Southern blots showed three, two and one copy numbers of *WLG4* in the hexaploid, tetraploid and diploid wheat genomes, respectively (data not shown). The results indicated that the common wheat genome possessed three homoeologous loci of the *WLG4* gene, one in each of the three components, A, B, and D genomes.

To study the expression pattern of *WLG4* in various organs of wheat, RT-PCR analysis was performed. *WLG4* transcripts were abundantly accumulated in SAM-containing embryos and young spikes, and floral organs, but not in leaf blade, sheath and ligule/auricle (Fig. 4). Maize *lg3* and *lg4* mRNA is expressed in apical regions but is not expressed in





**Fig. 3.** A phylogenetic tree of *WLG4* and its homologs based on their deduced amino acid sequences. Nei's genetic distances were calculated and the tree was constructed by UPGMA method.



**Fig. 4.** RT-PCR analysis of *WLG4*. Total RNAs were isolated from the indicated tissues of Chinese Spring. The numbers of PCR cycles are indicated at the right sides of the electrophoresis patterns. The actin gene is used as an internal control.

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## Research Information

# Class C MADS-box gene *AGAMOUS* was duplicated in the wheat genome

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### Abstract

Class C MADS-box gene is involved in specifying stamen and carpel identity during flower development in plant species. To obtain information about the molecular mechanism underlying floral organ formation in wheat, we identified two *AGAMOUS* (*AG*)-like MADS box genes, *WAG-1* (wheat *AGAMOUS-1*) and *WAG-2*. Phylogenetic analysis of *WAG-1* and *WAG-2*, together with class C MADS-box genes in barely, rice and maize, indicated that the monocot class C genes are classified into two clades, *WAG-1* clade and *WAG-2* clade. *Arabidopsis AG* is more close to *WAG-2* clade than *WAG-1* clade, suggesting that the genes in the former clade, wheat *WAG-2*, barley *HvAG1*, rice *OsMADS3*, and maize *ZMM2*, have more similar function to *AG*.

Flower development has been the subject of intensive study over the last decade, particularly in two dicot species, *Arabidopsis thaliana* and *Antirrhinum majus* (Jack 2004). These studies have provided a general understanding of the development of floral organs in higher plants and led to the production of the ABCDE model. This model postulates that floral organ identity is defined by five classes of homeotic gene, named A, B, C, D and E (Zahn et al. 2006). According to the ABCDE model, class A and E genes specify sepals in the first floral whorl, class A, B and E genes specify petals in the second whorl, class B, C and E genes specify stamens in the third whorl, class C and E genes specify carpels in the fourth whorl, and class D and E genes specify the ovule in the pistil. Cloning of ABCDE organ identity genes in *Arabidopsis* showed that they encode MADS-box transcription factors except for the class A gene, *APETALA2* (*AP2*). The class A MADS-box gene is *API*, the class B genes are *AP3* and *PISTILLATA* (*PI*), the class C gene is *AGAMOUS* (*AG*), and the class D gene is *SEEDSTICK* (*STK*). In *Arabidopsis*, the class E genes consist of four members, *SEPALLATA1* (*SEP1*), *SEP2*, *SEP3* and *SEP4*, which show partially redundant functions in

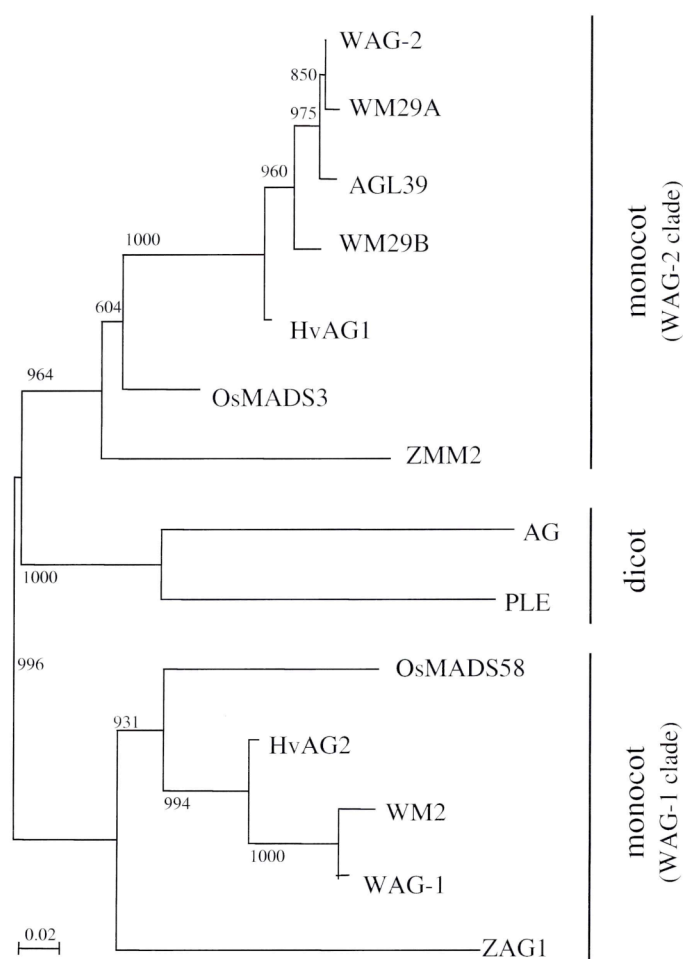
identity determination of petals, stamens and carpels.

Analysis of the ABCDE genes in monocot species such as rice suggests that the ABCDE model could essentially be extended to monocots except for the role of the class A gene (Kater et al. 2006; Yamaguchi and Hirano 2006). In wheat, it has been reported that the *API*-like gene *WAPI* (wheat *API*, sometimes called *VRN1*) (Murai et al. 1998; Murai et al. 2002) has no class A function but acts in phase transition from vegetative to reproductive growth (Murai et al. 2003). Using alloplasmic wheat line which shows pistillody, homeotic transformation of stamens into pistil-like structures, we identified a wheat *AP3* ortholog, *WAP3* (wheat *APETALA3*) (Murai et al. 1998), and two wheat *PI* orthologs, *WPI-1* (wheat *PISTILLATA-1*) and *WPI-2* (Hama et al. 2004) as the wheat class B genes. Furthermore, we identified two types of class E genes from wheat, *WSEP* (wheat *SEPALLATA*) and *WLHS1* (wheat *Leafy Hull Sterile 1*) (Shitsukawa et al. 2007). Our functional analysis indicated that *WSEP* is more likely to be orthologs of class E genes than *WLHS1*. As class C gene, we previously identified *WAG-1* (wheat *AG-1*) (Meguro et al. 2003), but its

function is unclear. Here we report the second wheat *AG*-like gene, *WAG-2*.

*WAG-2* was isolated from a wheat expressed sequence tag (EST) database (Ogihara et al. 2003). By screening all the EST contigs through a BLAST search, we identified a contig with high sequence similarity to *OsMADS3* in rice (Kang et al. 1995) and *ZMM2* in maize (Theissen et al. 1995), and named *WAG-2*. To inspect the relationship between wheat *AG*-like genes, *WAG-1* and *WAG-2*, and other members of the class C gene family in detail, the phylogenetic tree was reconstructed by using the amino acid sequences (Fig. 1). The phylogenetic tree indicated that the monocot class C gene family separated into two

groups, *WAG-1* clade and *WAG-2* clade. The *WAG-1* clade contains barley *HvAG2*, rice *OsMADS58* (Yamaguchi et al. 2006) and maize *ZAG1* (Mena et al. 1996), whereas the *WAG-2* clade consists of barley *HvAG1*, rice *OsMADS3* (Kang et al. 1995) and maize *ZMM2* (Theissen et al. 1995). This indicates that *AG* orthologs were duplicated in each monocot species. The phylogenetic tree also indicates that dicot class C genes, *Arabidopsis AG* and *Antirrhinum PLE*, are more close to *WAG-2* clade than *WAG-1* clade (Fig. 1). This suggests that the genes in the former clade, wheat *WAG-2*, barley *HvAG1*, rice *OsMADS3*, and



**Fig. 1.** Phylogenetic tree of deduced amino acid sequences of class C MADS-box genes. The phylogenetic tree was constructed by the neighbor-joining method using deduced amino acid sequences. The numbers at the nodes show bootstrap values after 1,000 replicates. The deduced amino acid sequences of the entire coding region were obtained from the DDBJ database: *WAG-1* (AB084577), *WAG-2* (AB465688), *WM2* (AM502863), *WM29A* (AM502898), *WM29B* (AM502899), *AGL39* (DQ512355) from wheat; *HvAG1* (AF486648) and *HvAG2* (AF486649) from barley; *OsMADS3* (L37528) and *OsMADS58* (AB232157) from rice; *ZMM2* (X81200) and *ZAG1* (L18924) from maize; *AG* (X53579) from *Arabidopsis*; and *PLE* (S53900) from *Antirrhinum*.

maize *ZMM2*, have more similar function to dicot class C genes. Functional analyses were performed in rice *AG* orthologs by using mutant and RNAi transgenic plants (Yamaguchi et al. 2006). The mutant and transgenic studies indicated that *OsMADS3* plays a more predominant role in inhibiting lodicule development and in specifying stamen identity, whereas *OsMADS58* contributes more to conferring floral meristem determinacy and to regulating carpel morphogenesis. It means that the duplicated class C genes in rice, *OsMADS3* and *OsMADS58*, show only partial conservation of function with the *Arabidopsis* class C gene, *AG*. Interestingly, carpel identity is determined by a YABBY gene named *DROOPING LEAF (DL)* in rice (Nagasawa et al. 2003; Yamaguchi et al. 2004).

Wheat (*Triticum aestivum* L.) is a hexaploid species with the genome constitution AABBDD that originated from three diploid ancestral species: the A genome came from *T. urartu*, the B genome from *Aegilops speltoides* or another species classified in the Sitopsis section, and the D genome from *Ae. tauschii* (Feldman 2001). Allopolyploidization leads to the generation of duplicated homoeologous genes (homoeologs), as opposed to paralogous genes (paralogs). Consequently, the hexaploid wheat genome contains triplicated homoeologs derived from the ancestral diploid species. In the wheat EST database, we can find four other *AG*-like genes in addition to *WAG-1* and *WAG-2*. Our phylogenetic analysis indicated that three genes, *WM29A*, *WM29B*, and *AGL39*, are classified into the *WAG-2* clade (Fig. 1). *WM29A* (identical with *TaAG-2A*) and *WM29B* (identical with *TaAG-2B*) were identified from cv. Chinese Spring (Paolacci et al. 2007), and *AGL39* was from cv. Nongda 3338 (Zhao et al. 2006). *WAG-2*, *WM29A* and *WM29B* should be three homoeologs in the A, B, or D genome of Chinese Spring wheat. Our phylogenetic analysis also indicated that *WAG-1* makes subclade with *WM2 (TaAG-1)* (Fig. 1). *WAG-1* was identified from cv. Norin 26 (Meguro et al. 2003), but *WM2* was from cv. Chinese Spring (Paolacci et al. 2007).

In conclusion, the wheat genome contains two *AG* orthologs, *WAG-1* and *WAG-2*, and at least in *WAG-2*, there are three homoeologs located in the A, B, or D genomes. Functional diversification of the three homoeologs should be examined in the future work.

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## Research Information

# Establishment of 14 wheat lines carrying telosomes of barley chromosome 7H

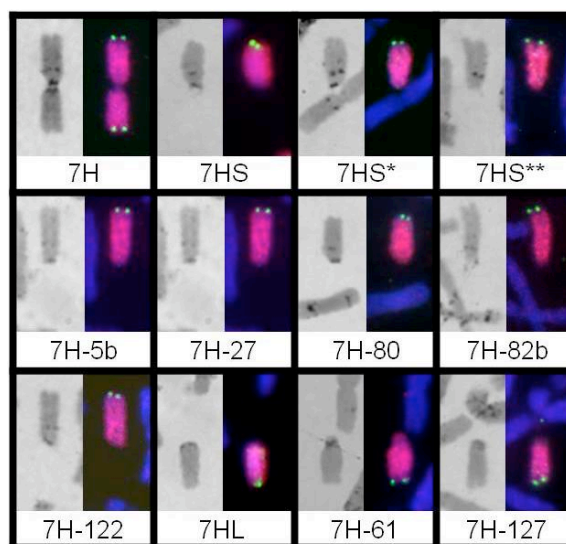
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The wheat (*Triticum aestivum* cv. Chinese Spring,  $2n=6x=42$ , genome formula AABBDD)–barley (*Hordeum vulgare* cv. Betzes,  $2n=2x=14$ , HH) addition lines (Islam et al. 1981) are useful to allocate genes and DNA markers to each barley chromosome. We have shown that the barley chromosomes added to wheat are susceptible to chromosome breakage genetically induced by gametocidal (Gc) chromosomes (Shi and Endo 1997, 1999, 2000; for review see Endo (2007)). To date, we have developed the barley chromosome dissection lines for chromosomes 3H (Sakai et al. 2009), 5H (Ashida et al. 2007), and 7H (Serizawa et al. 2001; Masoudi-Nejad et al. 2005; Nasuda et al. 2005b). These wheat lines carrying dissected barley chromosomes are powerful materials for the allocation of DNA markers to sub-arm regions of the individual barley chromosomes. For chromosome 7H, we have developed 19 stocks of Chinese Spring wheat carrying structurally modified 7H chromosomes (Nasuda et al. 2005a). The breakpoints of the modifications (deletions or translocations) are in various regions of chromosome 7H; eight each on the short and long arms, and three in the pericentromeric region. Two telosomes of the 7HS do not have centromeric repeats that are ubiquitous in the primary constrictions of all normal barley chromosomes (Nasuda et al. 2005b). PCR analysis of the EST markers in these lines allowed us to define seven and six bins on 7HS and 7HL, respectively.

Here, we report the establishment of additional 14 novel 7H-dissection lines that have breakpoints in the pericentromeric region. They were originally isolated from the 7H addition line of Chinese Spring carrying a Gc chromosome (2C) from *Aegilops cylindrica* (Shi and Endo 2000). In short, we made crosses between two disomic addition lines of common wheat carrying chromosome 7H (Islam et al. 1981) and a gametocidal (Gc) chromosome 2C from *Aegilops cylindrica* ( $2n=4x=28$ , DDCC) (Endo 1988),



**Fig. 1.** Photographs of the telocentric chromosomes. For each chromosome, C-band (left) and GISH/FISH (right) images are given. GISH signals with barley genomic DNA probe are in red, and FISH signals with the HvT01 probe are in green. Chromosomes are counter stained with DAPI (4',6-diamidino-2-phenylindole) and shown in blue. Line names possessing the telosomes are indicated below the images. Chromosomes 7H, 7HS, and 7HL of Islam et al. (1981) and 7HS\* and 7HS\*\* of Nasuda et al. (2005a) are also represented for comparison.

respectively. The  $F_1$  hybrids ( $21''+1'7H+1'2C$ ) were backcrossed to the 7H addition line to produce  $BC_1$  plants that were disomic for 7H and monosomic for 2C ( $2n=45$ ,  $21''+1'7H+1'2C$ ). The  $BC_1$  plants were then self-pollinated or cross-pollinated with CS to generate structural changes involving the 7H chromosome. We selected plants with telocentric chromosomes of 7H from the progeny of  $BC_1$  by

simultaneous genomic in situ hybridization (GISH) with barely genomic DNA probe and fluorescence in situ hybridization (FISH) with the subtelomeric repeat HvT01 (Belostotsky and Ananiev 1990). We identified the chromosome arm retained in the lines by C-banding. Wheat lines carrying telocentric chromosome were grown to maturity and crossed with normal Chinese Spring to isolate single modified chromosomes. So far, we obtained nine telosomes for the short arm of 7H (7H-5b, 7H-27, 7H-29St, 7H-37, 7H-80, 7H-82b, 7H-85St, 7H-105, and 7H-122) and five for the long arm (7H-6Lt, 7H-31Lt, 7H-61, 7H-83, and 7H-127). Most of the lines appear to be telocentric chromosomes in cytological analysis (Fig. 1). The telosomes for the short arm had the centromeric C-band diagnostic to 7HS, while the 7HS\*\* chromosome reported in Nasuda et al. (2005a) lacks it (Fig. 1). The telosomes of the long arm also had the centromeric C-bands. Chromosome 7H-61 appeared to be acrocentric in cytological observation, which was confirmed by the presence of a DNA marker locating on the short arm (Sakai et al. unpublished). All the lines are either homozygous or hemizygous for the critical 7H telosomes, so that they can be readily used for molecular mapping. These lines will be deposited in the National BioResource Project-Wheat, Japan (<http://www.shigen.nig.ac.jp/wheat/komugi/top/top.jsp>). Together with the 7HS and 7HL telocentric lines developed by Islam et al. (1981) and by Nasuda et al. (2005b), there are now 19 wheat lines carrying barley 7H telosomes available. These lines will be very useful for analysis of genes and markers in the centromeric region of 7H, where genetic recombination is greatly suppressed (Kunzel et al. 2000).

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## Review

# Marker assisted selection: a strategy for wheat improvement

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## Abstract

Wheat is most widely grown crop in the world, best adapted to temperate region and is a staple food of about 35% of the world population. Molecular markers have been introduced over last two decades, which has revolutionized the entire scenario of biological sciences. Traditionally, breeders have relied on visible traits to select improved varieties however; MAS rely on identifying marker DNA sequences that are inherited alongside a desired trait during the first few generations. Molecular markers are also considered as useful tools for pyramiding of different resistance genes and developing multi-line cultivars targeting for durable resistance to the disease. With the development of methodologies for the analysis of plant gene structure and function, molecular markers have been utilized for identification of traits to locate the gene(s) for a trait of interest on a plant chromosome and are widely used to study the organization of plant genomes and for the construction of genetic linkage maps. Breeders used molecular markers to increase the precision of selection for best trial combinations. With the development of AFLP and microsatellite marker systems, renewed studies are underway to analyze the genetic basis of many important traits in wheat. In light of the fresh challenges CIMMYT is giving emphasis on molecular breeding, functional genomics, deployment of transgenes for abiotic stresses etc., should get priority to maintain pace with time and growth. In near future, molecular markers can provide simultaneous and sequential selection of agronomically important genes in wheat breeding programs allowing screening for several agronomically important traits at early stages and effectively replace time consuming bioassays in early generation screens.

## Introduction

Wheat is most widely grown crop in the world, best adapted to temperate region and is a staple food of about 35% of the world population. Wheat is a major source of energy, protein and dietary fiber in human nutrition since decades. Wheat is a major crop contributing importantly to the nutrient supply of the global population. Since the beginning of agriculture, ten thousand years ago, the importance of varietal improvement is well known. In the ancient time, selection and introduction were commonly used method, since knowledge about use of hybridization, mutation and polyploidy were not in practice. Selections were made on the basis of physical appearance and to fulfill the requirements. Till 15th century, most of the varieties were developed either through primary selection or introduction. Breeders had the advantages of variability until 1970s and due to intensive crossing programmes; green revolution took place during 1967-1968. The development and

promotion of modern, high yielding varieties was the most important factor contributing to the enormous success of green revolution. During two generations leading up to the turn of the century the global population grew by 90 percent whilst food production expanded by 115 percent. The global food security is quite fragile, particularly when looking towards the middle of the century because of projected needs for human, animal and industrial uses. Global wheat production is expected to increase from nearly 600 million tons of present production level to around 760 million tons in 2020 with limited expansion of sown area. But now, due to non-existence of variability for the yield trait, it is not possible to develop a new variety either by selection or introduction. Selection based on knowing the location of the genes of interest gives the breeder a significant advantage, particularly for quantitative traits, where classical selection is done on the phenotype as a whole rather than on the underlying genetic determinants. Identification of

significant QTL marker associations forms the baseline for MAS of quantitative traits. Furthermore, other breeding methods like hybridization, which reshuffle existing variability in the population and tools like polyploidy and mutation bring challenges for allele in the available traits and are not possible to introduce a new trait from unrelated species. Molecular markers have been introduced over last two decades, which has revolutionized the entire scenario of biological sciences. DNA based molecular markers have acted as versatile tools and have found their position in various fields like taxonomy, physiology, embryology, genetic engineering etc. PCR brought about a new class of DNA profiling markers that facilitated the development of marker based gene tags, map based cloning of agronomically important genes, variability studies, phylogenetic analysis, synteny mapping, marker assisted selection of various genotypes. Molecular markers are identifiable DNA sequences found at specific locations on the chromosomes and transmitted by the standard laws of inheritance from one generation to next and considered as landmarks in the chromosome maps that can be useful to monitor the transfer of specific chromosome segments known to carry useful agronomic traits. Molecular markers have also provided an excellent opportunity to develop saturated genetic maps and to integrate genetic, cytological and molecular maps. Molecular markers are being used to tag specific chromosome segments bearing the desired gene(s) to be transferred into the breeding lines.

Traditionally, breeders have relied on visible traits to select improved varieties however; MAS rely on identifying marker DNA sequences that are inherited alongside a desired trait during the first few generations. Thereafter, plants that carry the traits can be picked out quickly by looking for the marker sequences, allowing multiple rounds of breeding to be run in quick succession (Kumar et al. 2007). Molecular markers make selection possible for breeders to combine desirable alleles at a greater number of loci and at earlier generations than is possible with conventional breeding methodologies. Molecular markers can circumvent more cumbersome, established pedigree breeding strategies and even generate plant genotypes unachievable by conventional methods (Young 1999). Molecular markers are required in a broad spectrum of gene screening approaches, ranging from gene-mapping with traditional 'forward-genetics' approaches through QTL identification studies to genotyping and haplotyping studies. Molecular markers are also considered as useful tools for pyramiding of different resistance genes and developing multi-line cultivars targeting for durable resistance to the disease (Xia et al. 2005).

Conventionally, plant breeding depends upon morphological/phenotypic markers for the identification of agronomic traits. With the

development of methodologies for the analysis of plant gene structure and function, molecular markers have been utilized for identification of traits to locate the gene(s) for a trait of interest on a plant chromosome and are widely used to study the organization of plant genomes and for the construction of genetic linkage maps. Molecular markers are independent from environmental variables and can be scored at any stage in the life cycle of a plant. Over the last several years, there has thus been marked increase in the application of molecular markers in the breeding programmes of various crop plants. Molecular markers not only facilitate the development of new varieties by reducing the time required for the detection of specific traits in progeny plants, but also fasten the identification of desired genes and their corresponding molecular markers, thus accelerating efficient breeding of resistance traits into wheat cultivars by marker assisted selection (MAS).

#### **Marker assisted selection**

MAS is a breakthrough technology that changes the process of variety cultivation from traditional field based format to a laboratory format. It is the use of molecular markers to track the location of genes of interest in a breeding programme. MAS is a form of indirect selection and most widely used application of DNA markers. Once traits are mapped a closely linked marker may be used to screen large number of samples for rapid identification of progeny that carry desirable characteristics. MAS is one of the most widely used applications of molecular marker technologies and one that plant breeders have been quick to embrace. Biotechnology have provided additional tools that do not require the use of transgenic crops to revolutionize plant breeding progress in molecular genetics has resulted in the development of DNA tags and marker assisted selection strategies for cultivar development. Several molecular marker types are available and they each have their advantages and disadvantages. Restriction fragment length polymorphisms (RFLPs) were the first to be developed (some 15 years) and have been widely and successfully used to construct linkage maps of various species, including wheat. With the development of the polymerase chain reaction (PCR) technology, several marker types emerged. The first of those were random amplified polymorphic DNA (RAPD), which quickly gained popularity over RFLPs due to the simplicity and decreased costs of the assay. However, most researchers now realize the weaknesses of RAPDs and use them with much less frequency. Microsatellite markers or simple sequence repeats (SSRs) combine the power of RFLPs (codominant markers, reliable, specific genome location) with the ease of RAPDs and have the advantage of detecting higher levels of polymorphism. The amplified fragment length polymorphism (AFLP) approach takes advantage of the PCR technique to

selectively amplify DNA fragments previously digested with one or two restriction enzymes (Hosington et al., FAO Document Repository). Later, microsatellite markers or SSRs (Simple Sequence Repeats) were developed, which took advantage over RAPD and RFLP. Playing with the number of selective bases of the primers and considering the number of amplification products per primer pair, this approach is certainly the most powerful in terms of polymorphisms identified per reaction.

The essential requirements for MAS in a plant-breeding program are as follows (Mohan et al. 1997):

- (a) Marker(s) should co-segregate or be closely linked (1 cM or less) with the desired trait.
- (b) An efficient means of screening large populations for the molecular markers should be available. A relatively easy analysis based on PCR technology is the best option.
- (c) The screening technology should have high reproducibility across laboratories, be economical to use and user friendly.

RFLPs, RAPDs and AFLPs do not fit the first requirement. However, techniques are available to turn them into user-friendly markers. RFLP clones can be sequenced and primers designed to amplify the DNA fragments are shown by hybridization to be polymorphic. However, the resulting STS or SCAR does not always turn out to be polymorphic and further manipulations are needed if this is the case. The amplified fragment is usually digested with one or two restriction endonucleases to detect small length differences, or the fragment from two or more cultivars is cloned and sequenced again to create ASAs. ASAs are usually based on single nucleotide differences. RAPD and AFLP fragments can be isolated from the gel, cloned and sequenced to generate STSs or SCARs. Attempts to generate such markers for wheat are neither always successful nor easily achieved. SSRs, on the other hand, if tightly linked to genes of interest are probably the most attractive markers since no further manipulations are needed for implementation. Despite the large number of markers for wheat genes listed in Table 2 few of those markers are close enough to the genes of interest to be useful in breeding applications.

Breeders used molecular markers to increase the precision of selection for best trial combinations. Variety developed by MAS are not considered genetically modified organisms (GMOs) and accepted by local and international market. Molecular marker aided selection methods have resulted in significant improvement in breeding efficiency by reducing trial and error aspect of breeding process and by allowing for time and cost savings. Molecular marker systems

will benefit from the constant increase in the integration of biotechnological production of segregating populations such as homozygous double haploids in wheat breeding cycles since, the major requirement of being co-dominant for molecular markers will disappear. Marker assisted selection (MAS) offers an opportunity to select desirable lines based on genotype rather than phenotype. Marker assisted selection is an invaluable tool for gene pyramiding (Bringing genes from different individuals together in one individual) and has been fairly successful for combining single gene traits.

Marker assisted selection (MAS) is based on the identification and use of markers, which are linked to the gene(s) controlling the trait of interest. By virtue of linkage, selection may be applied to the marker itself. The advantage consists in the opportunity of speeding up the application of the selection procedure. For instance, a character which is expressed only at the mature plant stage may be selected at the plantlet stage, if selection is applied to a molecular marker. Selection may be applied simultaneously to more than one character. Selection for a resistance gene may be carried out without needing to expose the plant to the pest, pathogen or deleterious agent. If linkage exists between a molecular marker and a quantitative trait locus (QTL), selection may become more efficient and rapid. The construction of detailed molecular and genetic maps of the genome of the species of interest is a prerequisite for most forms of MAS. However, the current cost of the application of these techniques is significant, and the choice of one cost technique rather than others may be dictated by factors. There are few examples of crop varieties in farmer's fields, which have developed through MAS.

Genetic resistance in wheat against diseases like leaf rust, stripe rust, stem rust, spot blotch, hill bunt etc., is generally governed by one, two or three genes and these genes can be tagged with any of the above DNA markers, specially which are based on PCR technology. In wheat, RFLPs have been used to map seed storage protein loci, loci associated with protein flour colour, cultivar identification, vernalization and frost resistance gene, intrachromosomal mapping of genes for dwarfing and vernalization, resistance to preharvest sprouting, quantitative trait loci (QTL) controlling tissue culture response, nematode resistance and milling yield. PCR based markers have been useful for characterization of genes for resistance against common bunt, powdery mildew, leaf rust resistance against hessian fly and Russian wheat aphid. RFLP, DNA sequencing, and a number of PCR-based markers are being used extensively for reconstructing phylogenies of various species. The techniques are speculated to provide path breaking information regarding the fine time scale on which closely related species have diverged and what sort of genetic variations are associated with species formation. Efforts are being made for studying the

**Table1.** Characteristics of the bread wheat genome that explain the slow progress in mapping as compared to a diploid, highly polymorphic species such as maize

Characteristic <sup>a</sup>	Wheat	Maize
Ploidy level	6x	2x
Number of chromosomes	21	10
Genome size (number of base pairs x 10 <sup>6</sup> )	16 000	4 500
Polymorphism level	Low	High
· RFLPs: probe x enzyme combinations (%)	20-30	80-85
· SSRs: primer pairs (%)	40-50	50-60
Repetitive sequences (%)	>80	60
To construct linkage maps of same density (15 markers/chromosome):		
Number of loci needed	315	150
Number of RFLP probes needed	1 000-1 500	200-250
Number of SSR primer pairs needed	700-800	250-300

<sup>a</sup>RFLP, Restriction Fragment Length Polymorphism; SSR, Simple Sequence Repeat.

genetic variation in plants to understand their evolution from wild progenitors and to classify them into appropriate groups (Jeffrey 1995). RFLP markers have proved their importance as markers for gene tagging locating and manipulating quantitative trait loci (QTL), in evolutionary studies for deducing the relationship between the hexaploid genome of bread wheat and its ancestors (Gill 1991). Specific markers like STMS (Sequence-tagged microsatellite markers) ALPs (Amplicon length polymorphisms) or STS markers have proved to be extremely valuable in the analysis of gene pool variation of crops during the process of cultivar development and classification of germplasm.

Wheat biotechnological research have been relatively slow, due to its ploidy level, the size and complexity of its genome, the very high percentage of repetitive sequences and low level of polymorphism (Table 1). Lack of genetic polymorphism in crops like wheat and soybeans and the consequent problems to identify molecular markers have been a major limitation to the impact of marker assisted selection (MAS) in wheat breeding. However, the identification of a high number of polymorphism in Single Sequence Repeats (SSR) should therefore, greatly enhance the potential to find molecular markers in wheat.

RAPDs emerged as a convenient and effective technique for tracing alien chromosome segments in translocation lines (Williams et al. 1990). RAPD markers provide a useful alternative to RFLP analysis for screening markers linked to a single trait within near isogenic lines and bulked segregants. He et al. (1992) reported the development of a DNA

polymorphism detection method by combining RAPD with DGGE (denaturing gradient gel electrophoresis) for pedigree analysis and fingerprinting of wheat cultivars. RAPD markers can be converted to more user-friendly Sequence Characterized Amplified Region (SCAR) markers, which display a less complex banding pattern. SCAR markers linked to resistance genes against fungal pathogens have been characterized in combination with RAPD and RFLP (Procunier et al. 1997; Myburg et al. 1998; Liu et al. 1999). In recent years, RAPD and other PCR based markers like Sequence Characterized Amplified Regions (SCAR), Sequence Tagged Sites (STS) and Differential Display Reverse Transcriptase PCR (DDRT-PCR) are increasingly being used for identification of desirable traits in wheat and related genera. These markers have been used in particular for disease resistance against viral and fungal pathogens and also for insect and nematode pests and have the potential of pyramiding of resistance genes for effective breeding programs. PCR based markers have been extensively characterized for genes of resistance against common bunt, *Tilletia tritici* (Demeke et al. 1996), powdery mildew, *Erysiphe graminis* (Hartl et al.; 1995; Qi et al. 1996), leaf rust, *Puccinia recondita* (Dedryver et al. 1996; Feuillet et al. 1995; Seyfarth et al. 1999), resistance against Hessian fly, *Mayetiola destructor* (Dweikat et al. 1994) and Russian wheat aphid, *Diuraphis noxia* (Myburg et al. 1998; Venter and Botha, 2000). SSRs or Microsatellites are more promising molecular markers for the identification and differentiation of genotypes within a species. The high level of polymorphism and easy handling has

made microsatellites extremely useful for different applications in wheat breeding (Devos et al. 1995; Roder et al. 1995; Bryan et al. 1997; Korzun et al. 1997; Roy et al. 1999). Microsatellites have also been used to identify resistance genes like *Pm6* from *Triticum timopheevii* (Tao et al. 1999) and *Yr15* from breadwheat (Chague et al. 1999) (Table 2).

#### **Use of molecular markers in wheat improvement programme: conservation of genetic resources**

Loss of genetic diversity has become a problem not only of the natural plant and animal population but also agriculturally important species. Ancient cultivars or landraces and wild relatives of domesticated species are being lost as modern varieties become adopted by farmers. This has led to calls for genetic conservation of crop germplasm (Frankel and Bennett 1970). Microsatellites are commonly used to study genetic relationships among genotypes within species because of their high level of polymorphism (Devos et al. 1995; Roder et al. 1995; Korzun et al. 1997). Microsatellites markers are currently used to identify genotypes Quantitative trait loci (QTLs) and genetic diversity (Medini et al. 2005). Knowledge of genetic diversity of wheat varieties is a prerequisite for the successful management of conservation programs. The first microsatellite in wheat possessed 279 microsatellites (Roder et al. 1998) by now a total of 1235 microsatellite loci were developed and mapped (Somers et al. 2004). Recently 101 microsatellite markers derived from expressed sequence tags EST-SSRs were added into a set of microsatellites (Gao et al. 2004).

#### **Resistance to biotic stress: gene pyramiding for multiple disease resistance**

Development of resistant varieties for single disease is not enough to save plant product and to feed growing population especially in developing countries. In India, there are number of constraints in the successful production of wheat like leaf and yellow rust, powdery mildew and spot blotch. These all diseases together toll heavy yield losses and put us in the situation to redesign our experiments to develop multiple pest resistant varieties in wheat. The grown varieties on the market do carry some known disease resistance genes against powdery mildew and rusts and low levels of quantitative resistance against leaf spot diseases. However, most of the resistance genes against rusts and powdery mildew are already broken down. In the sustainable agriculture, which is economical both for the farmer and nature, multiple disease resistance is an essential tool against pathogens attack beside cultural practices like crop rotation. With the biotrophic fungi like rusts and powdery mildew, the only solution is the durable disease resistance.

In late 1980's Fusarium head blight (FHB)

become a world wide problem. Earlier, it was sporadic and localized to China, South America and a few European countries. Varieties resistant to FHB such as Wuhan 1, 2 and 3 and Shangai 7 and 8 and Suzhoe wheats were the product of generous collaboration between various Chinese institutions. Fusarium head blight is a serious disease of wheat (*Triticum aestivum* L) in humid and semi humid areas of the world. Evaluation of Fusarium head blight resistance is time consuming, laborious and costly as the inheritance of resistance is complex phenomenon. The most recent biotic threat to global wheat production is Ug 99, a virulent race of stem rust which has emerged from Uganda and has been confirmed at widely distributed testing locations in Kenya and Ethiopia. Yield losses of up to 71 percent have been recorded under experimental conditions (Dixon 2003).

#### **Resistance to abiotic stress**

Traditional approaches at transferring resistance to crop plants are limited by the complexity of stress tolerance traits, as most of these are quantitatively linked traits (QTLs). The direct introduction of a small number of genes offers convenient alternative and a rapid approach for the improvement of stress tolerance. Although, present engineering strategies rely on the transfer of one or several genes that encode either biochemical pathways or endpoints of signalling pathways, these gene products provide some protection either directly or indirectly against environmental stresses. Drought is a major abiotic factor that limits crop productivity, thereby causing enormous loss.

Low temperature (LT) tolerance is a complex quantitative character that is expressed in anticipation of and during exposure of plants to temperatures that approach freezing. This environmentally reduced character is determined by a highly integrated system of structural and developmental genes that are regulated by environmentally responsive complex pathways. The superior LT-tolerance genes have been tagged using molecular markers that allow plant breeders to select hardy genotypes without having to wait for a test frost in the field (Fowler and Limin 2007).

#### **Quality traits**

Other than reporter genes, perhaps the most targeted trait for genetic engineering in wheat is quality. Seed storage proteins (SSP) are contained in the seed of higher plants. These proteins have been classified as albumins, globulins and glutenins on the basis of their solubility in solvents. The high molecular weight glutenin subunits (HMW-GS) genes in wheat are located on the long arm of the homeologous chromosomes 1A, 1B and 1D. Bread-making properties are particularly associated with variation at the *Glu-D1* and *Glu-A1* loci. The HMW-GS 1Ax1,

1Ax2, 1Dx5 and 1Dx10 have been shown to be associated with stronger dough, better elasticity and, hence, improved bread-making quality. Many elite wheat varieties lack the desired studies have demonstrated that the introduction of one or two HMW-GS genes results in a stepwise increase in dough elasticity. The transgenic lines produced so far have also demonstrated a very high level of expression and stability over several generations. This may imply that native genes are more tolerated by a plant genome subunits and, thus, many research groups are attempting to introduce these via genetic engineering (Shewry et al. 1995; Altpeter et al. 1996; Barro et al. 1997; Vasil and Anderson 1997). In addition to increasing the bread-making quality, altered amino acid composition of the SSP is feasible and could result in improved nutritional properties. For example the insertion of genes for proteins such as zeins or albumins, could lead to an increase in the desired amino acid. Other approaches are also being considered such as reducing the level of anti-nutritional factors and modifying starch and oil composition and content.

#### Challenges and future strategies in India

India's population of more than a billion is growing at a rate of around 1.8% per year, almost going parallel with the annual growth rate of cereals. Therefore, the estimated demand of wheat production for the year 2020 is around 109 million tones, which is 30 million tones more than the record production of 75 million tones harvested in the crop season 1999-2000. Since then, India is struggling to achieve the impressive figure of its record production. However, the ever increasing population has alarmed food security in India and attempts have been initiated to integrate modern technology tools in conventional breeding to improve the most important crops such as rice, wheat and legumes. There is little doubt that wheat has been a difficult species for the application of molecular genetics. The low level of polymorphism between elite varieties coupled with the hexaploid nature of the crop provides significant challenges for those attempting to develop molecular markers and to use them in genetic studies. With the development of AFLP and microsatellite marker systems, renewed studies are underway to analyze the genetic basis of many important traits in wheat.

Future challenges include developing strategies for reducing the cost per assay, acquire more desirable markers to further complement the efforts of wheat breeders as well as evaluating emerging technologies to increase throughput with reduced cost. Integrating molecular breeding efforts in a few target national program partners also is considered an important challenge. One of the major concerns of wheat researchers is to make Indian wheat globally competitive by reducing the cost of cultivation and increasing farmer's profitability. With the availability of detailed information regarding the location and function of gene(s) encoding for useful traits, scientists in future will be well equipped for efficiently creating varieties with exact combinations of desirable traits. However, genetic transformation will remain a significantly important tool for understanding gene functions and for testing the utility of new sequences. In near future, crop varieties could be tailor-made to meet both local consumer preferences and the demands of particular environment or niche. CIMMYT, Mexico has played an important role in strengthening the Indian wheat programme since the advent of Green Revolution. In light of the fresh challenges CIMMYT is giving emphasis on molecular breeding, functional genomics, deployment of transgenes for abiotic stresses etc., should get priority to maintain pace with time and growth. In near future, molecular markers can provide simultaneous and sequential selection of agronomically important genes in wheat breeding programs allowing screening for several agronomically important traits at early stages and effectively replace time consuming bioassays in early generation screens. Although, the potential of biotechnology has often been exaggerated, a high level of optimism is clearly justified for its use in the improvement of wheat. Undoubtedly, functional genomics, as it is now termed, will revolutionize the way in which plant breeding is undertaken in the future. Basic research is leading to an improved understanding of the genetic mechanisms operating within a plant in response to the diverse stresses that it is exposed to, as well as the overall production of biomass and grain. The challenge for developing countries is to tap as much of this emerging technology as possible.

**Table 2.** Published markers for important genes in wheat (Source: Hoisington, FAO Document repository)

Trait <sup>a</sup>	Locus <sup>b</sup>	Source <sup>c</sup>	Marker <sup>d</sup>	Chromosome	Reference
Disease resistance					
Leaf rust	<i>Lr1</i>	<i>Triticum aestivum</i>	RFLP/STS	5DL	Feuillet et al. (1995)
	<i>Lr3</i>	<i>T. aestivum</i>	RFLP	6BL	Parker et al. (1998)

	<i>Lr9</i>	<i>Aegilops umbellulata</i>	RAPD/STS	6BL	Schachermayr et al. (1994)
			RFLP		Autrique et al. (1995)
	<i>Lr10</i>	<i>T. aestivum</i>	RFLP/STS	1 AS	Schachermayr et al. (1997)
	<i>Lr13</i>	<i>T. aestivum</i>	RFLP	2 BS	Seyfarth et al. (1998)
	<i>Lr18</i>	<i>T. timopheevii</i>	N-band	5BL	Yamamori (1994)
	<i>Lr19</i>	<i>Thinopyrum</i>	RFLP	7DL	Autrique et al. (1995)
			Isozyme		Winzeler et al. (1995)
	<i>Lr20</i>	<i>T. aestivum</i>	RFLP	5 AL	Parker et al. (1998)
	<i>Lr23</i>	<i>T. turgidum</i>	RFLP	2BS	Nelson et al. (1997)
	<i>Lr24</i>	<i>Agropyron elongatum</i>	RFLP	3DL	Autrique et al. (1995)
			RAPD/STS		Schachermayr et al. (1995)
			RAPD/SCAR		Dedryver et al. (1996)
	<i>Lr25</i>	<i>Secale cereale</i>	RAPD	4BL	Procurier et al. (1995)
	<i>Lr27</i>	<i>T. aestivum</i>	RFLP	3BS	Nelson et al. (1997)
	<i>Lr29</i>	<i>Ag. elongatum</i>	RAPD	7DS	Procurier et al. (1995)
	<i>Lr31</i>		RFLP	4BL	Nelson et al. (1997)
	<i>Lr32</i>	<i>Ae. tauschii</i>	RFLP	3DS	Autrique et al. (1995)
	<i>Lr34</i>	<i>T. aestivum</i>	RFLP	7DS	Nelson et al. (1997)
	QTL	<i>T. aestivum</i>	RAPD/RFLP	7BL, 1DS	William et al. (1997)
Suppressor	<i>SuLr23</i>		RFLP	2DS	Nelson et al. (1997)
	<i>Sr2</i>	<i>T. turgidum</i>	RFLP/STS	3BS	Johnston et al. (1998)
	<i>Sr5</i>	<i>T. aestivum</i>	RFLP	6DS	Parker et al. (1998)
Stem rust	<i>Sr9e</i>	<i>T. aestivum</i>	RFLP	2BL	Parker et al. (1998)
	<i>Sr22</i>	<i>T. monococcum</i>	RFLP	7AL	Paull et al. (1995)
	<i>Sr36</i>	<i>T. timopheevii</i>	RFLP	2BS	Parker et al. (1998)
Stripe rust	<i>Yr15</i>	<i>T. dicoccoides</i>	RFLP/RAPD	1BS	Sun et al. (1997)
	<i>Pm1</i>		RFLP	7AS	Ma et al. (1994)
			RFLP		Hartl et al. (1995)
			RAPD-STS		Hu et al., 1997
	<i>Pm2</i>		RFLP	5D	Ma et al., (1994); Hartl et al. (1995)
			RFLP, STS		Mohler and Jahoo (1996)
	<i>Pm3</i>		RFLP	1A	Ma et al. (1994)
Powdery mildew			RFLP		Hartl et al. (1993)
	<i>Pm4a</i>		RAPD		Li et al. (1995)
	<i>Pm4b</i>		AFLP		Hartl et al. (1998)
	<i>Pm12</i>	<i>Ae. speltoides</i>	RFLP	6B/6S	Jia et al. (1996)
	<i>Pm18</i>		RFLP	7AL	Hartl et al. (1995)
	<i>Pm21</i>	<i>Haynaldia villosa</i>	RAPD	6VS, 6AL	Qi et al. (1996)
	<i>Pm25</i>	<i>T. monococcum</i>	RAPD	1A	Shi et al. (1998)

Suppressor	SuPm8		Storage protein	1AS	Ren et al. (1996)
Wheat streak mosaic virus	<i>Wsm1</i>	<i>Ag. elongatum</i>	STS		Talbert et al. (1996)
Common bunt	<i>Bt-10</i>		RAPD		Demeke et al. (1996)
	Ut-X (T10)		RFLP/RAPD-	-	Procunier et al. (1997)
Loose smut			STS		
	T19		Antibody	6A	Knox and Howes (1994)
Eyespot	<i>Pch1</i>		RFLP/Isozyme	7DL	Chao et al. 1989
	<i>Pch2</i>	<i>T. aestivum</i>	RFLP	7AL	de la Pena et al. (1997)
Tan spot	QTL		RFLP	1AS, 2DL, 4AL,	Faris et al. (1997)
Fusarium scab	QTL	<i>T. aestivum</i>	AFLP/RFLP	3BS, 2AL, 6BS, 4BL	Anderson et al. (1998)
Karnal bunt	QTL	<i>T. turgidum</i>	RFLP	3BS, 5AL	Nelson et al. (1998)
Pest resistance					
	<i>H3,5,6,9, 10,11,12, 13,14,16,17</i>		RAPD	1A, 5A	Dweikat et al. (1997)
Hessian fly	<i>H9</i>		RAPD	-	Dweikat et al. (1994)
	<i>H21</i>	<i>S. cereale</i>	RAPD	2RL	Seo et al. (1997)
	<i>H23, H24</i>	<i>Ae. tauschii</i>	RFLP	6D, 3DL	Ma et al. (1993)
	<i>H27</i>	<i>Ae. ventricosa</i>	Isozyme	4M <sup>V</sup>	Delibes et al. (1997)
	<i>Cre1</i>	<i>T. aestivum</i>	RFLP-STS	2BL	Williams et al. (1994, 1996)
Cereal cyst nematode	<i>Cre2</i>		RFLP	6BL	Paull et al. (1998)
	<i>Cre3</i> (Ccn-D1)	<i>Ae. tauschii</i>	RAPD	2DL	Eastwood et al. (1994)
Stress tolerance					
Cadmium uptake			RAPD		Penner et al. (1995)
Aluminium tolerance	<i>Alt2</i>		RFLP	4D	Luo and Dvorak (1996)
			RFLP	4DL	Riede and Anderson (1996)
Drought induced ABA			RFLP	5A	Quarrie et al. (1994)
Na <sup>+</sup> /K <sup>+</sup> discrimination	<i>Kna1</i>	<i>T. aestivum</i>	RFLP	4D	Alien et al. (1995)
			RFLP	4DL	Dubcovsky et al. (1996)
Quality traits					
Kernel hardness	<i>Ha</i>		RFLP	5D	Nelson et al. (1995a)

	<i>Hn</i> , QTL		RFLP	5DS, 2A, 2D, 5B, 6D	Sourdille et al (1996)
Grain protein	QTL	<i>T. turgidum</i>	RFLP	4BS, 5AL, 6AS, 6BS, 7BS	Blanco et al. (1996)
High protein		<i>T. dicoccoides</i>	ASA	6B	Humphreys et al. (1998)
LMW glutenin		<i>T. turgidum</i>		1B	D'Ovidio and Porceddu (1996)
HMW glutenin	<i>Glu-D1-1</i>	<i>T. aestivum</i>	ASA	1DL	D'Ovidio and Anderson (1994)
Flour colour			RFLP/AFLP	7A	Parker et al. (1998)
Other traits					
Pre-harvest sprouting	QTL	<i>T. aestivum</i>	RFLP	-	Anderson et al. (1993)
Vernalization	<i>Vrn1</i>		RFLP	5AS	Galiba et al. (1995); Nelson et al. (1995a); Korzun et al. (1997); Kato et al. (1998)
	<i>Vrn3</i>		RFLP	5DS	Nelson et al. (1995a)
Photoperiod	<i>Ppd1</i>	<i>T. aestivum</i>	RFLP	2DS	Worland et al. (1997)
	<i>Ppd2</i>	<i>T. aestivum</i>	RFLP	2BS	Worland et al. (1997)
Dwarfing	<i>Rht8</i>		SSR	2DS	Korzun et al. (1998)
	<i>Rht 12</i>		SSR	5AL	Korzun et al. (1997)
Fertility restoration	<i>Rf1, Rf3</i>		RFLP	6BS, 1BS	Ma and Sorrells (1995)
	<i>Rf4</i>				
Meiotic pairing	<i>ph1b</i>		RFLP/STS	5BL, 5BL	Gill and Gill (1996)
	Deletion		AFLP/STS		Qu et al. (1998)

<sup>a</sup>ABA, Abscisic Acid; K<sup>+</sup>/Na<sup>+</sup>, potassium/sodium; LMW, Low Molecular Weight; HMW, High Molecular Weight

<sup>b</sup>QTL, Quality Trait Locus

<sup>c</sup>*Ae. tauschii* (syn. *T. tauschii*)

<sup>d</sup>RFLP, Restriction Fragment Length Polymorphism; STS, Sequence Tagged Site; RAPD, Random Amplified Polymorphic DNA; SCAR, Sequence Characterized Amplified Region; AFLP, Amplified Fragment Length Polymorphism; ASA, Allele Specific Amplicon; SSR, Simple Sequence Repeat

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## Topics on Wheat Genetic Resources

# National BioResource Project Wheat: Overview

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Wheat includes bread wheat, which is used to make bread and udon noodles, and macaroni wheat, which is used to make macaroni and other forms of pasta. The NBRP Wheat project stores and supplies wild species, landraces, and experimental strains of wheat and related species. It also collects and stores wild species and landraces that have not yet been archived, and stores and supplies EST and TAC clones of wheat. The project is implemented by the Graduate School of Agriculture, Kyoto University (core facility) and the

Kihara Institute for Biological Research, Yokohama City University (sub-facility). The Wheat Subcommittee, which is organized by scientists who conduct wheat research in Japan, supports the core team's work (Fig. 1). The project's wheat resources can be requested online. The project members are also focusing on the characterization of DNA markers for use in gene isolation, and will release the resulting data in the NBRP section of the KOMUGI database.

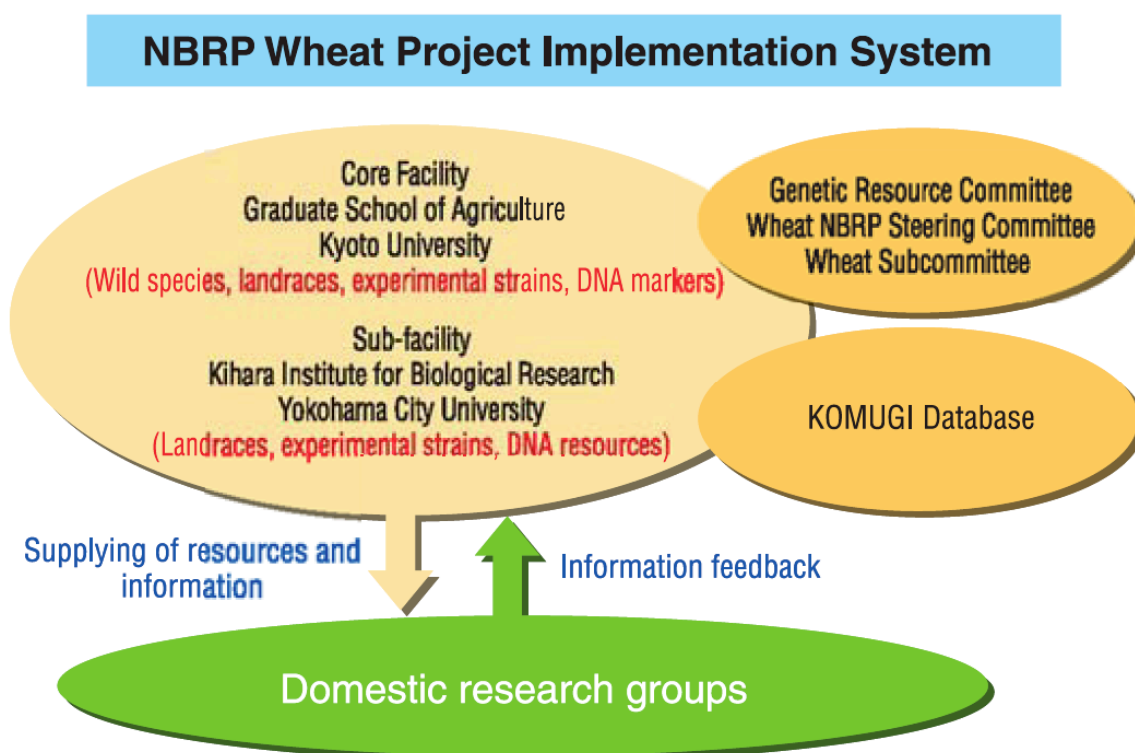


Fig. 1. NBRP Wheat Project Implementation System



## Topics on Wheat Genetic Resources

# The report of National Bioresource Project-Wheat II. Seed resources, 2007

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The second stage of NBRP-Wheat has started in April 2007. In this second stage, the activity in seed resources is focused on the maintenance and distribution of the genetic stocks collected during the first stage of NBRP-Wheat. Achievements during April 2007 to March 2008 are reported here.

**Maintenance of seed resources:** Regeneration of total of 748 strains was finished early in summer 2007. Seventy-five landraces and 14 experimental lines of tetraploid wheat were grown in Kihara Institute for

Biological Research, Yokohama City University (KIBR) and 659 strains consisting of several wild species and landraces were grown in Graduate School of Agriculture, Kyoto University. In autumn 2007 1195 strains were sown, 354 at KIBR and 941 at Graduate School of Agriculture, Kyoto University.

**Distribution of seed resources:** Total of 689 strains have been distributed to various researchers and institutions throughout the world (Table 1).

**Table 1.** Number of seed stocks distributed

Code (Institution) *	No. of strains distributed
KT (KIBR)	10
KU (MOZUME)	246
LPGKU	410
TACBOW	23

\* KIBR: Kihara Institute for Biological Research, Yokohama City University, LPGKU and MOZUME: Graduate School of Agriculture, Kyoto University, TACBOW: Faculty of Agriculture, Tottori University.



## Topics on Wheat Genetic Resources

# A report of the project “Polymorphism survey among hexaploid wheat and its relatives by DNA markers” granted by the National Bioresource Project-Wheat, Japan

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The National Bioresource Project-Wheat (NBRP-Wheat), launched by the Japanese government in 2002, is aimed to maintain and distribute seed stocks and DNA clones of wheat. Additionally to its primary roles in handling seed stocks and DNA clones, the second-term NBRP-Wheat, started in 2007, features the collection and characterization of DNA markers. The objectives of the project are; (1) to make the resources of the NBRP-Wheat more valuable for molecular studies by addition of genotype information, and (2) to find a set of DNA markers that is suitable for detecting polymorphisms among wheat samples. The outline of the current project is illustrated in Fig. 1.

Simple sequence repeat (SSR) is a PCR-based method to detect polymorphisms. SSR is widely used in wheat sciences such as linkage mapping, QTL mapping, marker-assisted selection, and phylogenetic studies. We obtained primer information of more

than 3000 SSR markers from the publications and public databases. Avoiding duplications, we synthesized a total of 2545 primer sets (1861 SSR primers and 684 STM primers). The markers consisted primary of *barc* (Song et al. 2005), *cfa* and *cfb* (Guyomarc'h et al. 2002; Sourdille et al. 2004), *gdm* (Pestsova et al. 2000), *gwm* (Röder et al. 1995, 1998), *hbg*, *hbe*, and *hbd* (Torada et al. 2006), and *wmc* (Gupta et al. 2002; Somers et al. 2004) markers. Primers for the STM markers were synthesized according to Hayden et al. (2002, 2004).

The 48 plant lines subjected to polymorphism survey are listed in Table 1. The lines to be tested include; eight *Aegilops* species with representative diploid genomes, *Triticum monococcum*, *T. boeoticum*, *T. urartu*, *T. durum*, *T. spelta* and 31 hexaploid wheat accessions. We also took samples of *Hordeum vulgare*, *H. spontaneum*, and *Secale cereale* as outgroup species.

Plant DNA was isolated from young fresh leaves with the DNeasy Plant Mini Kit (QIAGEN) and quantified by a spectrophotometer. PCR reaction was carried out with the iCycler (BioRad). On our hands, the optimum PCR condition was achieved by addition of betaine and DMSO into the reaction mixture at the final concentrations of 1 M and 3%, respectively. The PCR products were subjected to electrophoresis in a capillary gel-electrophoresis apparatus HAD-GT12 (eGene, now available from QIAGEN) with a gel cartridge GCK-5000. Electrophoretic patterns were analyzed using the software Biocalculator supplied with the electrophoresis apparatus.

We started our survey with those SSR markers mapped by Somers et al. (2004). So far, we obtained amplification profiles of 717 SSR primers for the 36 lines, and 222 SSR markers for the rest 12 lines (in total, we obtained 28476 amplification profiles).

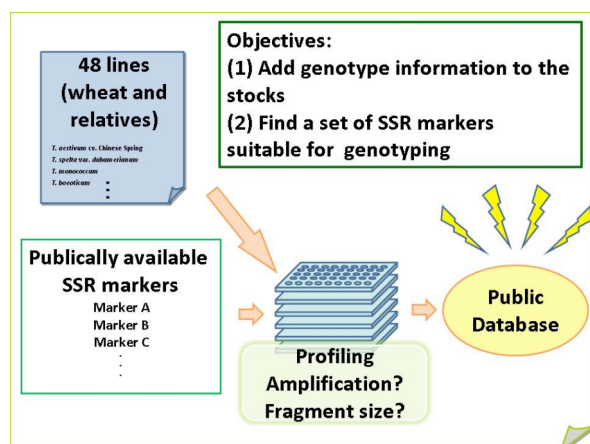


Fig. 1. An outline of the project “Polymorphism survey among hexaploid wheat and its relatives by DNA markers”.

**Table 1.** The plant materials subjected to the polymorphism survey.

Aegilops species (8 lines)

*Ae. speltoides*, *Ae. longissima*, *Ae. tauschii*,  
*Ae. umbellulata*, *Ae. caudata*, *Ae. uniaristata*,  
*Ae. comosa*, *Ae. mutica*

Diploid wheat (3 lines)

*T. monococcum*, *T. boeoticum*, *T. Urartu*

Tetraploid wheat (1 line)

*T. durum* cv. Langdon

Hexaploid wheat (except *T. aestivum*) (1 line)

*T. spelta* var. *duhamerianum*

*T. aestivum* accessions (31 lines)

[non-Japanese] (9 lines)

Opata 85, Synthetic, Chinese Spring,  
Timstein, Hope, Cheyenne, Sumai 3,  
KS831957, U24

[Japanese] (22 lines)

Hokkai 240, Kanto 107, Norin 26, Norin 61,  
Hokushin, Chihokukomugi, Nanbukomugi,  
Fukuhokomugi, Zenkojikomugi, Kitanokaori,  
Minaminokaori, Haruyokoi, Hanamanten,  
Kitahonami, Kinuiroha, Saikai 165,  
Ayahikari, Minaminokomugi, Chogokuwase,  
Abukumawase (winter type),  
Nobeokabozukomugi, Tamaizumi

Outgroups (4 lines)

*Secale cereale* cv. Imperial, *Hordeum vulgare* cv.  
Betzes, *H. vulgare* cv. Harunanijo, *H.*  
*spontaneum* H602

Due to the technical limitations of the capillary gel-electrophoresis, we encounter difficulties in detecting polymorphism with small differences in fragment lengths. We are planning to test at least 2000 markers on the 48 samples by the end of year 2010, resulting in 96000 PCR profiles. We are in discussion with Dr. Yukiko Yamazaki at National Institute of Genetics, Japan for the method of presentation of our data through the KOMUGI web site (<http://www.shigen.nig.ac.jp/wheat/komugi/top/top.js> p). We welcome your valuable comments for establishing a user-friendly database.

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Nakamura, Z. Nishio, K. Sato, T. Terachi, H. Tsujimoto, and Y. Yoshimura. We especially thank Dr. Masaya Fujita for his generous coordination of the lines used in Japanese breeding programs. We express our gratitude to Drs. G. Ishikawa, K. Kato, Y. Matsuoka, and S. Takumi for their valuable advices. This work is supported by the National Bioresorce Project-Wheat, the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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## Topics on Wheat Genetic Resources

# Annual report of National Bioresource Project-Wheat II. DNA resources, 2007

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In the first year of the NBRP-Wheat II, DNA resource section is continuously promoting the systematic collection of cDNA clones and ESTs in addition to the stock of DNA resources collected in the first stage. The sequences of the full length cDNA clones were also collected supported by the NBRP Genome program in 2007. Achievements during April 2007 to March 2008 are reported here.

**Collection of DNA resources:** Total of ca. 85000 ESTs from four cDNA libraries were sequenced. These libraries were constructed from seedling of Norin 4 wheat under various conditions, namely seedlings treated with water (control), these infected with wheat blast fungus at 23 °C, these incubated at 23 °C after infection with oat blast fungus, and these incubated at 27 °C after infection with oat blast fungus. Independent 5387 full length cDNA clones were selected and completed their sequencing. 14 TAC clones, including certain genes, were screened.

**Maintenance of DNA resources:** Approximately 40000 cDNA clones and 14 TAC clones were newly stored. EST information was processed to open at KOMUGI.

**Distribution of DNA resources:** 138 cDNA clones were distributed, of which 89 clones were sent to domestic researchers and 49 clones to foreign institutes. 14 TAC clones were sent to domestic researchers.

**Related activity of DNA resources:** The oligo-DNA microarray harboring 38k gene probes of common wheat were developed under the collaboration with Agilent Technology and distributed to domestic researchers. TILLING lines of Chinese Spring wheat were produced and all genomic DNA extracted from the TILLING lines were stored for use.



## Meeting Reports

# The Third Triticeae Meeting of Japan, 2008

## Kazuhiro Sato\*

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The third Triticeae Meeting of Japan was held at Research Institute for Bioresources, Okayama University December 6, 2008. More than 100 researchers and students participated in the meeting (Fig. 1). Two special lectures by Profs. Kazuyoshi Takeda and Kazuhiko Noda were given under co-organization with Sanyo Breeding Workshop. Two plenary talks from barley and wheat, other five talks from recent Triticeae scientific activities were presented. Thirty posters with short oral explanation were presented mainly by young scientists. Summary of presentations were described below.

Another meeting was organized by Okayama University at the same place. The title was "Identification of important genes and their application to breeding in barley". Recent activities of barley genome research and gene isolation were presented. Invited lectures were also presented by Dr. Nils Stein, IPK, representing International Barley Sequencing Consortium and Dr. Etienne Paux, INRA, for wheat chromosome 3B analysis. These two-day activities reviewed the advancements of Triticeae science internationally and gave good opportunity for young scientists and researchers in other fields to know the current and ongoing activities on Triticeae science.

## ABSTRACTS & TITLES

### Oral Presentation

#### **01. Genetical studies on barley ; A special lecture**

##### **Kazuyoshi Takeda**

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1) A brief history of Sanyo branch of Japanese Society of Breeding was introduced.

2) History of Barley Germplasm Center

In 1942 the first scientific paper on the barley genetics was published by Ryuhei Takahashi, a leader of

Japanese barley genetics. Barley germplasm preservation project was funded by Monbusho in 1967. Barley Germplasm Preservation Lab. was established in 1979, and it was expanded as Barley and Wild Plant Resource Center in 1997. In Barley division, we preserve ca. 14,000 germplasm accessions, DNA resources and their on-line database systems.

3) Selected topics of barley genetic studies

Semi-dwarf "uzu": In 1942 Dr. R. Takahashi published the first paper concerning the varietal variation of coleoptile length, i.e. long vs. short. The short type was named "uzu" after the dwarf mutant of the morning glory. The uzu varieties were distributed in the south part of Japan and the Korean peninsula. In China, uzu varieties were grown more than one million ha in 1960~1970s. The trait is controlled by a single nucleotide mutation caused an amino-acid change which resulted in brassinosteroid insensitivity.

Diazinon sensitivity: Varietal variation of the sensitivity to an insecticide diazinon was found. The sensitivity is caused by a single dominant gene named *Diz* located on chromosome 7H. Out of 5,560 varieties tested, 708 were sensitive to diazinon and exclusively distributed in west of India. Varieties with *Diz* in east of China were western origin. Thus, *Diz* is an important marker trait to study the phylogeny of barley.

Deep seeding tolerance: Because the topsoil in semi-arid regions is very dry. Seeds are usually sown deep. Thus, varieties have to be capable to emerge from the depth. The deep-seeding tolerance in more than 4,000 barley accessions was evaluated. The varietal variation was very large and deep seeding tolerant varieties elongate the first internode which usually does not occur when seeds were sown shallow. The major QTLs for the deep seeding tolerance were found on chromosome 5H and 7H.

## **O2. Nucleotide-substitution rate of the B-genome donor species of wheat; a special lecture**

**Kazuhiko Noda**

Research Institute for Bioresources, Okayama University, Kurashiki, 710-0046, Japan

Phylogenetic studies of hexaploid wheat, *Triticum aestivum* (AABBDD), have shown that *Aegilops speltoides* (SS) is a candidate species for the B-genome donor; however, this finding remains controversial. It has been suggested that the high rate of nucleotide change just after polyploidisation and during diversification of *Ae. speltoides* may cause ambiguity in predicted B-genome ancestry. We compared 29 orthologous *dihydroflavonol-4-reductase* (*DFR*) genes from the *Triticum-Aegilops* complex, the synthetic wheat, barley and maize, and estimated the amount of nucleotide change among these *DFRs* through the use of the Molecular Evolutionary Genetics Analysis (MEGA) software. *Ae. speltoides* (SS) was the closest species to the B and G genomes of tetraploid (*T. turgidum* [AABB]), *T. araraticum* [AAGG] and hexaploid wheat (*T. aestivum* [AABBDD]). However, there were more nucleotide differences between *Ae. speltoides* and the B and G genomes than between *T. urartu* (AA) and the A genome of tetraploid and hexaploid wheats or between *Ae. squarrosa* (DD) and the D genome of hexaploid wheat. Polyploidisation does not appear to induce a high rate of nucleotide change because no significant change was detected in the synthetic hexaploid wheat. The substitution-rate heterogeneity test showed that a high rate of nucleotide change occurred in *Ae. speltoides* compared with nucleotide changes in the other species of the *Sitopsis* section (SS). The high rate of nucleotide change in *Ae. speltoides* might have caused ambiguity in predicted B-genome ancestries.

## **O3. Multiple molecular events responsible for barley domestication**

**Takao Komatsuda**

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Early cultivators of barley (*Hordeum vulgare* ssp. *vulgare*) selected a spike phenotype with non-brittle rachis. Two genes responsible for non-brittle rachis (*btr1* and *btr2*) were genetically detected more than 50 years ago, and existence of the two genes allowed Dr. R. Takahashi, Okayama University, Kurashiki, to suggest at least two independent origins of cultivated barley. A physical map covering the *btr1* and *btr2* genes has been constructed for the isolation of these genes. Non-brittle rachis was followed by a six-rowed spike that stably produced three times the usual grain

number during domestication. Six-rowed spike 1 (*Vrs1*) was cloned. *Vrs1* encoded a homeodomain leucine zipper I-class protein (HD-ZIP I), a potential transcription factor. *Vrs1* is expressed only in lateral spikelet primordia of the early developmental stage. Analysis of plenty of mutant lines allowed the gene identification. In addition to many mutational events detected at the coding sequence of *Vrs1* gene, mutational event at the regulatory regions of *Vrs1* was suggested in five mutant lines. Three independent origins of six-rowed barley were caused by loss-of-function mutation of the homeobox gene, while another origin showed no DNA changes throughout the coding region of the *Vrs1* gene leaving its mutational event unknown. Report on the isolation of naked caryopsis (*nud*) indicated a single origin of naked barley of the world. Seed dormancy is controlled by rather complex genetic system, QTL, and SD1 and SD2, the major QTL, would be targets for gene isolation. Origin of vernalization non-requirement (*Vrn-H1* or *Sgh2*) was reported to be multiple, while photoperiod insensitive gene *ppd-H1* was suggested to be of a single origin. Molecular cloning of genes responsible for domestication and adaptation may allow inferring developmental process of cultivated barley as an important crop.

## **O4. Genome resources and functional genomics in common wheat**

**Yasunari Ogihara**

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Because wheat harbors huge genome size and is characteristic of its polyploidy nature, we have conducted comprehensive collections of expressed sequence tags (ESTs) in common wheat for several years. Up to now, 55 cDNA libraries derived from tissues during the wheat life cycle as well as stress-treated tissues were constructed. Several thousand colonies were randomly selected from each of these cDNA libraries and their inserts were sequenced from both of 5'- and 3' ends. Sequence data of about one-million ESTs are now available. These ESTs were grouped into about 90 thousands homoeologs and 38 thousands gene clusters with CAP3/phrap and BLAST methods. These contigs were estimated to cover more than 90 % of expressed wheat genes. By computing ESTs, correlated expression patterns of genes across the tissues (Virtual Display: VD) were monitored including stress-treated tissues. Thus, genes specifically induced and/or suppressed by certain stresses were able to be selected from the VD. These genes were annotated with the BLAST search. Furthermore, by using these contigs, we had constructed oligo DNA microarray spotting

38K wheat gene probes under collaboration with Agilent Co. Ltd. This array was applied to select salt (NaCl) responsive genes in common wheat. We also promoted systematic survey and sequencing of the wheat full-length cDNA clones to carry out gene annotation in the wheat genome and functional genomics of wheat. These functional genomics data of wheat should provide powerful tools for wheat breeding.

## **O5. Molecular mechanisms controlling adhesion of hulls to caryopses in barley.**

### **Shin Taketa**

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Typical barley cultivars have caryopses with adhering hulls at maturity, known as covered (hulled) barley. However, a few barley cultivars are a free-threshing variant called naked (hulless) barley. The covered/naked caryopsis is controlled by a single locus (*nud*) on chromosome arm 7HL. Such differentiation in caryopsis types is unique to the barley crop. By means of positional cloning, it was concluded that an ERF (ethylene response factor) family transcription factor gene controls the covered/naked caryopsis phenotype. This conclusion was supported by (1) fixation of the 17-kb deletion, harboring the ERF gene, among all 100 naked cultivars studied, (2) two x-ray induced *nud* alleles with a DNA lesion at a different site, each affecting the putative functional motif, and (3) gene expression strictly localized to the testa. Available results indicate the monophyletic origin of naked barley in the world. The *Nud* gene has homology to the *Arabidopsis* *WIN1/SHN1* transcription factor gene, whose deduced function is control of a lipid biosynthesis pathway. Staining with a lipophilic dye (Sudan Black B) detected a lipid layer on the pericarp epidermis only in covered barley. It is suggested that, in covered barley, the contact of the caryopsis surface, overlaid with lipids to the inner side of the hull, generates organ adhesion. Details were reported in our recent publication, Taketa *et al.* PNAS 4062-4067(2008).

## **O6. “The origin of bread wheat” revisited - fieldwork, diversity analysis, and QTL mapping**

### **Yoshihiro Matsuoka**

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Bread wheat (*Triticum aestivum* L. ssp. *aestivum*) has a hexaploid genome (genome constitution AABBDD) derived from a hybrid cross between a cultivated form

of tetraploid *Triticum* wheat (*T. turgidum* L., genome constitution AABB) as the female parent and a wild diploid species *Aegilops tauschii* Coss. (genome constitution DD) as the male parent. The hybrid cross is supposed to have produced a fertile triploid F<sub>1</sub> hybrid (genome constitution ABD) that spontaneously set hexaploid seeds by producing unreduced gametes in male and female gametogenesis. Bread wheat represents cases in which the genetic relationships between crops and their relatives are known. Important questions, however, remain concerning the genetic and ecological mechanisms that underlie the early stages of the evolution of bread wheat. How often does *Ae. tauschii* grow in the *T. turgidum* fields? Under what environmental conditions does the *T. turgidum*-*Ae. tauschii* hybrid cross successfully take place? Which *Ae. tauschii* population has the ability to produce the fertile triploid F<sub>1</sub> hybrid with *T. turgidum*? To what extent is the fertility of the triploid F<sub>1</sub> hybrid genetically controlled? In the last four years, we addressed these questions by (1) fieldwork to examine the current ecological status of *Ae. tauschii* and *T. turgidum* in northern Iran, (2) diversity analysis to examine the natural variation for fertile *T. turgidum*-*Ae. tauschii* F<sub>1</sub> hybrid formation, and (3) QTL mapping to examine the genetic basis of the *T. turgidum*-*Ae. tauschii* F<sub>1</sub> hybrid fertility. In this talk, I will summarize the results of those studies and discuss advances in understanding the evolution of bread wheat.

## **O7. Low temperature stress signal pathways in wheat**

### **Fuminori Kobayashi**

Research Fellow of the Japan Society for the Promotion of Science, Plant Genome Research Unit, National Institute of Agrobiological Sciences, Kan-non-dai 2-1-2, Tsukuba, Ibaraki 305-8602, Japan

Low temperature induces and/or enhances expression of numerous *Cor* (cold-responsive)/*Lea* (late embryogenesis abundant) genes, and accumulated COR/LEA proteins promote the development of freezing tolerance by protecting cellular components. In wheat, a number of low temperature-inducible *Cor/Lea* genes have been isolated, and genetic maps of major loci and QTLs affecting cold/freezing tolerance have been constructed. *Fr-1* (*Frost resistance-1*) and *Fr-2* are well known to be major loci determining winter hardiness. *Fr-1* controls development of freezing tolerance and *Cor/Lea* gene expression through transcriptional activation of the *CBF* gene family, tandemly located on the *Fr-2* loci. *Wcbf2*, *Wdreb2* and *Wlip19* out of four cold-responsive transcription factor genes identified in our previous studies were transcriptionally activated through *Fr-1* under low temperature conditions.

Transgenic tobacco plants expressing either these transcription factors showed a significant increase in freezing tolerance. The direct interaction between the transcription factors and wheat *Cor/Lea* promoters was *in vivo* confirmed using the wheat cultured cells and the transgenic tobacco plants. These results indicate that WCBF2, WDREB2, WABI5 and WLIP19 act as transcriptional regulators to activate *Cor/Lea* gene expression under low temperature conditions. In other words, various stress-responsive transcription factors cooperatively function in development of cold/freezing tolerance in wheat. On the other hand, it is well known that abscisic acid (ABA) also regulates responses to environmental stress. In fact, two wheat lines, ‘EH47-1’ (ABA-less-sensitive mutant) and ‘Mutant ABA 27’ (ABA-hypersensitive mutant), showed significantly increased freezing tolerance comparing with their parental lines, suggesting that ABA sensitivity is associated with determination of freezing tolerance level in wheat. Four QTLs for ABA sensitivity were identified on chromosomes 1B, 2A, 2B and 6D in common wheat seedling. Because of limited information about functional roles of the ABA signal pathways on activation of the cold-responsive genes, further studies should be required to confirm functional relationship between each of the identified QTLs and cold/freezing tolerance.

#### **O8. What is the real model for flowering gene network in wheat and barley?**

##### **Koji Murai**

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In cereal crops such as wheat and barley, heading time associated with the timing of floral transition (flowering) is an important character because of its influence on the adaptability to various environmental conditions. Several orthologous genes have been identified for flowering regulation in wheat and barley, suggesting that the common regulatory mechanisms are involved in the flowering of wheat and barley. Among the genes for flowering regulation, *VRN1* (identical with *WAP1*), *VRN2* and *FT* were identified to play the central roles in flowering. *VRN1* and *FT* are activators of flowering, but *VRN2* is a repressor. Two different models for flowering gene network have been presented by Jorge Dubcovsky’s group and Ben Travaskis’s group. According to our mutant and transgenic studies, the third model can be presented. In our model, *VRN1* is upstream of *FT* and possibly acts with *CO* to activate *FT* expression under LD conditions. Like in *Arabidopsis* and rice, FT proteins could be the florigen that moves from the leaves into the SAMs to determine floral meristem identity in

wheat and barley. *VRN2* is down-regulated by vernalization, and suppresses the *VRN1* expression. Furthermore, *VRN2* is down-regulated by *FT*. Consequently, a feedback triangle of *VRN1-FT-VRN2* could be the central mechanism in the wheat flowering. Our model can explain why *VRN1* represses *VRN2* expression, and why *VRN2* is epistatic to *VRN1*. It is important to notice that the gene interactions shown in the model are events occurring in leaves not in SAMs.

#### **O9. Wheat Research In India: Current Status**

##### **H. S. Balyan**

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Wheat is one of the most important grain crops of India, which is second to only rice in production. India ranks first in terms of area under wheat cultivation and second in terms of production and consumption after China. Current wheat production in India contributes ~8% to the total world’s wheat production. To maintain self-sufficiency in food grains and to meet the projected demands of wheat grain in 2025, the annual production of wheat and rice needs to increase by 2 mt every year. With increasing urbanization and changes in life style, the demand for diversified end-products of wheat is also rising steadily. To meet these challenges, wheat research currently is focused on development of wheat varieties with improved (a) yield potential, (b) tolerance to biotic and abiotic stress, (c) water use efficiency, (d) micronutrient (Zn and Fe) composition, and (d) end-use quality. An overview of the efforts to meet the above challenges through classical plant breeding including the use of alien species and molecular approaches will be presented. Significant efforts made towards development and use of molecular markers for introgression and pyramiding the genes/QTL for different traits including leaf rust resistance and grain quality will be discussed in some details with emphasis on the work done during the past few years in our laboratory. The role of India in wheat genome sequencing will also be presented.

#### **Poster Presentation**

**P1.** Sanae Shimada (Dep. Biosci., Fukui Pref. Univ.) Why a wheat line Cho-gokuwase is so early heading?

**P2.** Naoki Shitsukawa (Dep. Biosci., Fukui Pref. Univ.) Transcription variant in wheat class B MADS-box gene *WAP3*.

**P3.** Hiroko Kinjo (Dep. Biosci., Fukui Pref. Univ.) Is *WFUL2* is a wheat class A MADS box gene?

- P4.** Yuki Fujiwara (Dep. Biosci., Fukui Pref. Univ.) Heading characters in synthetic hexaploid wheat and its ancestral species.
- P5.** Megumi Suzuki (Dep. Biosci., Fukui Pref. Univ.) Epigenetic expression mechanism in the homoeologs of wheat class E MADS-box gene *WLHS1*.
- P6.** Tonooka, T., E. Aoki, T. Yoshioka (Natl. Inst. Crop Sci., NARO) Breeding of barley with new useful quality in NICS.
- P7.** Matsuoka, Y.<sup>1</sup>, S. Takumi<sup>2</sup>, T. Kawahara<sup>3</sup> (<sup>1</sup>Fukui Pref. Univ., <sup>2</sup>Laboratory of Plant Genetics, Graduate School of Agricultural Science, Kobe Univ., <sup>3</sup>Graduate School of Agriculture, Kyoto Univ.) Flowering time diversification and dispersal in central Eurasian wild wheat *Aegilops tauschii* Coss.: genealogical and ecological framework.
- P8.** Matsunaka, H., M. Chono, M. Seki (Natl. Inst. Crop Sci., NARO) Screening of new genotype of *R-A1* and its pedigrees.
- P9.** Seki, M., M. Chono, H. Matsunaka (Natl. Inst. Crop Sci., NARO) Breeding of near-isogenic lines for *Ppd-1*, controlling photoperiodic response in wheat.
- P10.** Kawakami, K.<sup>1</sup>, T. Kawahara<sup>2</sup>, H. Nishida<sup>1</sup>, Y. Akashi<sup>1</sup>, K. Kato<sup>1</sup> (<sup>1</sup>Grad. Sch. Natural Sci. Tech., Okayama U., <sup>2</sup>Grad. Sch. Agr., Kyoto U.) Molecular genetic analysis of vernalization response gene in wild species of diploid wheat.
- P11.** Sibai, S., Y. Omura, Y. Akashi, H. Nishida and K. Kato (Grad. Sch. Natural Sci. Tech., Okayama U.) Evolution and diversification of spring type wheat - Geographical variation in the sequence variation at *Vrn-D1* locus.
- P12.** Matsumoto, A.<sup>1</sup>, T. Tonooka<sup>2</sup>, D. Ishihara<sup>1</sup>, E. Aoki<sup>2</sup>, T. Yoshioka<sup>2</sup>, Y. Akashi<sup>1</sup>, H. Nishida<sup>1</sup> and K. Kato<sup>1</sup> (<sup>1</sup>Grad. Sch. Natural Sci. Tech., Okayama U., <sup>2</sup>Natl. Inst. Crop Sci.) Genetic diversity of heading time related genes of barley and its relation with heading date in the field.
- P13.** Mizuno, N., S. Takumi (Lab. Plant Genetics, Grad. Sch. Agr. Sci., Kobe Univ.) Transcriptome profiling of hybrid necrosis in hybrids between emmer wheat and *Aegilops tauschii*.
- P14.** Kajimura, T., N. Mizuno, S. Takumi (Lab. Plant Genetics, Grad. Sch. Agr. Sci., Kobe Univ.) Identification of senescence-associated ESTs in common wheat.
- P15.** Kurahashi, Y., A. Terashima, S. Takumi (Lab. Plant Genetics, Grad. Sch. Agr. Sci., Kobe Univ.) Comparative study of ABA sensitivity in D-genome-varied synthetic hexaploid wheat lines.
- P16.** Takumi, S.<sup>1</sup>, E. Nishioka<sup>1</sup>, Y. Matsuoka<sup>2</sup> (<sup>1</sup>Lab. Plant Genetics, Grad. Sch. Agr. Sci., Kobe Univ., <sup>2</sup>Fukui Pref. Univ.) Intraspecific variation of *Aegilops tauschii* revealed by spikelet morphology.
- P17.** Garg, M., H. Tanaka, H. Tsujimoto (Fac. Agr., Tottori Univ.) Exploitation of the variation of *Agropyron elongatum* and *Aegilops geniculata* for improvement of bread making quality.
- P18.** Ishii, T., T. Ueda, H. Tanaka, H. Tsujimoto (Grad. Sch. Agr., Tottori Univ.) Behavior of pearl millet chromosomes in Triticeae-pearl millet hybrid embryos.
- P19.** Sakata, T., Y. Taguchi, H. Tanaka, H. Tsujimoto (Grad. Sch. Agr., Tottori Univ.) Production of wheat lines with the fragments of alien chromosome by pollen irradiation method.
- P20.** Tanaka, K., M. Shimada, H. Tanaka, H. Tsujimoto (Grad. Sch. Agr., Tottori Univ.) Application of TILLING to detection of polymorphism in SSR markers in wheat.
- P21.** Ban, T.<sup>1,2</sup>, H. Buerstmayr<sup>3</sup>, J.A. Anderson<sup>4</sup> (<sup>1</sup>KIBR, Yokohama City Univ., <sup>2</sup>CIMMYT, <sup>3</sup>IFA-Tulln, Austria, <sup>4</sup>UMN, USA) Consensus map of Fusarium head blight resistance QTL in wheat.
- P22.** Arifi, M.<sup>1,2</sup> (<sup>1</sup>MAIL ARIA, Afghanistan, <sup>2</sup>KIBR, Yokohama City Univ.) Wheat production and breeding techniques in Afghanistan.
- P23.** Manickavelu A.<sup>1</sup>, K. Kawaura<sup>1</sup>, N. Yahiaoui<sup>2</sup>, B. Keller<sup>2</sup>, Y. Ogihara<sup>1</sup> (<sup>1</sup>KIBR, Yokohama City U., <sup>2</sup>Inst. Plant Biology, University of Zurich) Identification of new O-methyltransferase gene in common wheat induced by fungal pathogen.
- P24.** Kouyama S, K. Kawaura, Y. Ogihara (KIBR, Yokohama City U.) Analyses of global gene expression patterns in wheat during the allopolyploidization course.
- P25.** Saito M., M. Isshiki, K. Kawaura, Y. Ogihara (KIBR, Yokohama City U.) Molecular analyses of seed storage proteins in bread wheat.
- P26.** Takaku M., K. Kawaura, Y. Ogihara (KIBR, Yokohama City U.) Variation of the spike morphology in TILLING lines of common wheat.
- P27.** Hoshikawa A., Y. Tetsu, K. Kawaura, Y. Ogihara (KIBR, Yokohama City U.) Functional

analysis of wheat NAC transcription factors in response to salt stress.

**P28.** Yasumoro, M., K. Kawaura, Y. Ogihara (KIBR, Yokohama City U.) Molecular study on regulation systems of gene expression in hexaploid wheat.

**P29.** Tetsu Y., M. Saito, A. Hoshikawa, K. Kawaura, Y. Ogihara (KIBR, Yokohama City U.) Production of

transgenic lines suppressed NAC genes with RNA in *Triticum aestivum*.

**P30.** Naruse T., A. Hoshikawa, K. Kawaura, Y. Ogihara (KIBR, Yokohama City U.) Genetic diversity of bread wheats to salt response in Xin Jiang and Gan Su.



**Fig. 1.** The third Triticeae Meeting of Japan - Participant Group Photo

(Editorial comment)

Dr. K. Sato summarized the meeting report of "The Third Triticeae Meeting of Japan, 2008" held on December 6 in Kurashiki, Okayama, Japan. We circulate the abstracts of oral presentations and the titles of poster presentations as edited by Dr. Sato.



## Meeting Reports

# Meeting Report of the 11th International Wheat Genetics Symposium

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The 11th International Wheat Genetics Symposium (IWGS) was held during 24th and 29th of August, 2008 in Brisbane Convention Centre, Brisbane, Australia. About 400 wheat researchers and/or breeders got together in the third largest city of Australia. Forty attendants were from Japan. This is the second most attendants, and the Australians were the first (158 people). On the demands of Australian breeders, issues on wheat breeding, especially tolerance or resistance against biotic as well as abiotic stresses were main topics. The symposium put weight on the application field. While, reflecting recent innovations of genomics in wheat, genomics section was set up. The wheat genetics and breeding are coming of new era, so called genome breeding.

However, polyploidy is a great wall to carry out genome breeding. Another barrier is huge genome size of wheat, in which repetitive sequences disturb genome research for wheat genetics and breeding. Nevertheless, genome sequencing of chromosome 3B has been started as a model case. Tools for functional genomics have also developed in wheat. These useful tools must bring us new insights of wheat genetics and breeding.

In the business session, Yokohama, Japan had been selected as the site of the 12th IWGS. We, the local organizing committee, are planning to have an up-to-date symposium, and looking forward to having many attendants in Yokohama.





## Others

# 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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Editorial Office will inform authors of the status of their manuscript via e-mail as quickly as possible. The "eWIS online submission system" offers easy and straightforward web-based submission procedures. For text writing, Microsoft Word is recommended. Manuscripts should be double-spaced and page-numbered starting from the title page. Do not use line numbers. Figures including illustrations, photographs and color plates should be submitted as JPEG files. PDF is not an acceptable file format.

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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

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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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References should be cited in the text by the author(s) and year, and listed at the end of the text with the names of authors arranged alphabetically. When an article has more than two authors, only the first author's name should appear, followed by "et al.", in the text. The references should be formatted as follows.

Journal articles:

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](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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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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