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Chromosome 3B of Chinese Spring wheat is not a prerequisite for the gametocidal action of the $Gc3-C1$ gene

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Abstract

In order to test the hypothesis that chromosome 3B of Chinese Spring wheat is required for the action of a gametocidal gene $Gc3-C1$, we examined fertilities of the monosomic for chromosome 3C from $Aegilops$ triuncialis in the wheat background with and without the 3B chromosome. The fertilities were very low irrespective of the presence of chromosome 3C of Chinese Spring. This result indicated that the 3B chromosome of Chinese Spring is not the prerequisite for $Gc3-C1$ to exert its Gc action.

Key words: wheat, gametocidal gene, Igcl
Fig. 1. The electrophoretic patterns of amplification products of the TNAC1684 marker on a 1% agarose gel. Lanes 1 and 6 are 1 Kb DNA ladder (Invitrogen). Lane 2, 3, 4, and 5 corresponds to CS, N26, CS+3C"(3B") and N26+3C", respectively. Note that a 3C specific amplification in lanes 4 and 5 at around 750 bp (indicated by an arrow with “3C”).

Since the first discovery of IgI on 3B*N26 by Tsujimoto and Tsunewaki (1985), the fertility of the critical lines has been examined always in the presence of 3B*. The objective of this study is to test the hypothesis that the 3B* chromosome is a prerequisite for the Gc function of Gc3-C1.

First, we confirmed that a PLUG (PCR-based Landmark Unique Gene) marker TNAC1648, which is assigned to the most distal bins of the group-3 short arm (3AS4-0.45-1.00, 3BS8-0.78-1.00, 3DS4-0.59-1.00) in hexaploid wheat (Ishikawa et al. 2009), generates a chromosome 3C specific amplification product (Figure 1). We adopted two ISBP (Insertion site based polymorphic) markers cfp1125 and cfp3310, which locate in the bin C-3BS1-0.33 and C-3BL2-0.22, respectively (Paux et al. 2008). We used these three markers to identify chromosomes 3C and 3B in the following crossing experiments, instead of performing cytological observation.

The key line to generate Gc monosomic status with and without 3B CS is the disomic substitution line CS+3C"(3B*CS), where chromosome 3C from Ae. triuncialis disomically substitute chromosome 3B*. In order to test our new hypothesis, we made the following crosses CS monosomic 3B (2n = 41) x CS+3C"(3B*CS), nullisomic 3B tetrasomic 3D (NT3B3D, 2n = 42) x CS+3C"(3B*CS), CS x CS+3C"(3B*CS), and N26 x CS+3C"(3B*CS). In the first cross, the progenies segregate for chromosome 3BCS to produce 2n = 41 plants monosomic for 3C and nullisomic for 3B*CS or 2n = 42 plants doubly monosomic for 3C and 3B*CS. The plants were selected by PCR experiment for further fertility test. In the second cross, all progenies would be 2n = 42 monosomic for 3C and trisomic for 3D and nullisomic for 3B*CS. By these

| Table 1. Fertilities of the lines monosomic for the Gc chromosome 3C, and monosomic or nullisomic for wheat chromosome 3B |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Cross           | Plant ID        | Presence of    | Presence of    | Fertility       |
|                 |                 | chromosome 3C  | chromosome 3B  | (%)             |
|                 |                 | as indicated   | as indicated   |                 |
|                 |                 | by             | by             |                 |
|                 |                 | TNAC1648       | cfp1125        | cfp3310         |                 |
| CS monosomic 3B| 1               | +              | +              | +               | 0.0             |
|                 | 2               | +              | +              | +               | 0.0             |
|                 | 3               | +              | +              | +               | 0.0             |
|                 | 4               | +              | -              | -               | 0.0             |
|                 | 5               | +              | -              | -               | 0.0             |
|                 | 6               | +              | -              | -               | 0.0             |
| NT3B3D          | 1               | +              | -              | -               | 1.7             |
|                 | 2               | +              | -              | -               | 5.0             |
|                 | 3               | +              | -              | -               | 0.0             |
| CS+3C"(3B")    | 3               | +              | -              | -               | 0.0             |
| CS x            | 1               | +              | +              | +               | 0.0             |
| CS+3C"(3B")*   |                 |                 |                 |                 |                 |
| CS+3C"(3B")*   |                 |                 |                 |                 |                 |

“+” and “-” mean amplified and not amplified, respectively. The fertilities are the averages of three spikes.

* For these control lines, fertility was calculated from the seed set of the three plants (one spike per plant).
crosses and selection with the PCR marker, we established experimental conditions to compare nullisomic and monosomic status for 3B<sub>CS</sub>, both carrying 3C chromosome monosomically. The progenies were grown in an incubator (23°C and 16 h light) until reaching maturity. Seed set of the first and second florets in the self-pollinated spikes were counted as follows; number of seeds was counted until the numbers florets reached to 20 from the middle part of spikes. The fertility of a single spike was calculated by dividing the number of seeds by 20, and the fertility of a given plant was defined by an average of fertility rate of the three spikes (Table 1). The controls, 2n = 42 plant doubly monosomic for 3B<sub>CS</sub> and 3C, obtained from the cross between CS x CS+3C"(3B<sub>CS</sub>)" and 2n = 42 plant doubly monosomic for 3B<sub>N26</sub> and 3C obtained from the cross between N26 x CS+3C"(3B<sub>CS</sub>)" showed strikingly different fertility; low (0%) and high (78.3%), respectively. Other plants showed low fertilities (0-5.0%) regardless of the presence/absence of chromosome 3B<sub>CS</sub>. This observation clearly indicated that absence of chromosome 3B<sub>CS</sub> did not interfere the Gc action of Gc3-C1. Therefore, we concluded that chromosome 3B of Chinese Spring wheat is not a prerequisite for the Gc action of Gc3-C1.

References
A tip to generate a radiation hybrid map of wheat chromosome: How could we connect linkage groups separated at the pericentromeric region on chromosome 6B?

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Abstract
In the process of radiation hybrid mapping of chromosome 6B, we encountered a problem that a map was divided into to linkage groups. The cause of splitting map was that number of molecular markers conserved in the pericentromeric region among the group-6 chromosomes was less than other regions. For the chromosomal region reported for structural modifications, it is better to test non-syntenic markers as well in radiation hybrid mapping.

Key words: wheat, chromosome 6B, radiation hybrid mapping, chromosome structural changes

Bread wheat (Triticum aestivum L., 2n = 6x = 42, AABBDD) is a hexaploid with large and complex genome, which makes decoding of the genome sequence very difficult. Since 2005, International Wheat Genome Sequencing Consortium (IWGSC) has been steering the projects of whole genome sequencing of bread wheat. It aims to finish draft genome sequencing by the protocol relying on the BAC-based physical maps. It is reported that the BAC-based physical maps are still under construction for nine of 21 wheat chromosomes (as reported in the IWGS home page at the following URL; http://www.wheatgenome.org/Projects/IWGSC-Read-Wheat-Projects). In the IWGSC framework, the Japanese team led by the National Institute of Agrobiological Sciences is in charge of physical mapping and genome sequencing of the wheat chromosome 6B. We are taking part of the projects to develop molecular markers and to construct chromosome maps of 6B.

For sequencing of the wheat genome, construction of an accurate global physical (BAC-contig) map anchored to a high-resolution chromosome map is prerequisite (Gill et al. 2004). However, the construction of high-resolution genetic map of wheat chromosome by the conventional methods based on genetic recombination is not realistic because of unevenly distributed recombination events along chromosomes. In general, proximal half of chromosome arms are inert with respect to genetic recombination during meiosis (Lukaszewski and Curtis 1993; Akhunov et al. 2003). Radiation hybrid (RH) mapping is one of the mapping methods that do not rely on meiotic recombination. It uses radiation-induced chromosome breakage instead of meiotic recombination as a method of marker segregation for mapping. RH maps, which
have been first adopted in human, were developed in plant species such as maize (Kynast et al. 2004) and cotton (Gao et al. 2004). In wheat, high-resolution RH maps have been made for chromosomes 1D (Kalavacharla et al. 2006) and 3B (Paux et al. 2008). Especially, the RH map of 3B was effectively used for anchoring BAC-contigs of chromosome 3B to the chromosome map (Paux et al. 2008). In our study, we established the RH population for high-resolution RH map that would be used for BAC-anchoring for chromosome 6B sequencing (Watanabe et al., in preparation). Here, we report troubleshooting in construction of a RH map so that two separated linkage groups could be connected by a molecular marker.

We followed the protocol established by Yamano et al. (2010) for RH mapping. The detail of RH mapping procedure will be published elsewhere (Watanabe et al., in preparation). Shortly, the nullisomic 6B tetrasomic 6A (NT6B6A) line of Sears (1954) was crossed with the irradiated Chinese Spring (CS) wheat pollen that are prepared by irradiation of gamma ray (15 Gy) to mature spikes of CS by a Cs-137 Gammacell 40 Exactor (courtesy of Dr. Junya Kobayashi, Radiation Biology Center, Kyoto University). We sampled leaves from 461 progeny and extracted DNA by using DNeasy Mini Plant Kit (QIAGEN). We tested 21 SSR markers and four PLUG (PCR-based Landmark Unique Gene) marker (Ishikawa et al. 2009). Presence/absence of markers was analyzed by electrophoresis QIAxcel equipped with the screening kit (QIAGEN). We also employed 18 deletion lines (Endo and Gill 1996) for RH mapping with the 25 markers. The analysis of RH data and construction of RH map were carried out by CarthaGene (www.inra.fr/mia/T/CarthaGene/). CarthaGene generated a RH map subdivided to two linkage groups, corresponding to the short and long arms of chromosome 6B, regardless incorporation of the marker genotypes of the deletion lines. This is due to lack of markers in the pericentromeric region although we analyzed the most proximal PLUG markers (6BS-CEN and 6BL-CEN) for both arms that kept syntenic localization in the bin-maps among the group-6 chromosomes (Ishikawa et al. 2010). Then, we added a PLUG marker TNAC3947 which located in the non-syntenic region on the group-6 proximal short arms (Saito et al., unpublished data). This marker successfully united two separate linkage groups by the order 6BS-CEN - (117.9 cR) - TNAC3947 - (69.3 cR) - gwm608. The distance between 6BS-CEN and TNAC3947 is the longest among the marker intervals in the RH map (in total 715.6 cR). These results indicate that the selection of molecular markers is very important in RH mapping. If we have only tested the syntenic markers, we could not have connected to linkage groups. Eventually, a pericentromeric inversion is reported for chromosome 6B (Qi et al. 2006).

Although the RH mapping approach is not influenced by the genetic recombination that is strongly inhibited in the pericentromeric regions of wheat chromosomes, the resolution of RH map in the region also tends to be less than those in the distal region. Since the centromere is the crucial chromosomal domain for transmission of the critical chromosome to the offspring, the pericentromeric regions are retained in the RH populations more than other regions (Watanabe et al., in preparation). Therefore the pericentromeric regions are co-retained but not co-deleted in the RH population, which leads to the low resolution in a RH map. In our study, use of the deletion stocks enhanced this tendency. Most deletions showed very simple marker presence/absence patterns (Fig. 1), indicating that the most deletion lines are of so-called deficiency type (missing only the terminal region of chromosomes). This observation supports our previous report that chromosome deletions by the gametocidal system (for review see Endo 2007).
are the simple deletions (Joshi et al. 2013; Sakaguchi et al., in preparation). Thus, use of deletion stocks forced pericentromeric region co-retained more frequently than other regions. The take home message from our study is that when you construct a RH map, it is better to try multiple markers in the region where you are aware of the genome restructuring that disturbed the syntenic relationship among homoeologous chromosomes.

References


Research Opinion & Topics

A new high yielding, disease resistant winter wheat variety for Afghanistan

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Abstract
Afghanistan depends on irrigated wheat for 70 to 90% of its wheat harvest. Winter wheat has traditionally been an important contributor to Afghan wheat production; however share of winter wheat varieties in national wheat certified seed production has come down from about 20% to 10% mainly because of lack of suitable resistant varieties. Bamyan 013 was introduced in Afghanistan through winter wheat observation nursery in 2006-07 and was later tested in yield evaluation trials for four years at three winter wheat locations. The variety was found suitable for Afghan conditions as it out-yielded the popular winter wheat checks like Bezostaya, Solh 02 by a margin ranging from 8% to 31% during four years testing and was therefore released for cultivation in August 2013.

Introduction
Wheat is Afghanistan’s staple food crop covering about 2.5 million hectare producing slightly over five million tons in 2012 (Agriculture Prospect Report, 2012) at an average productivity of 1.99 tons per hectare. However, Afghanistan wheat production fluctuates owing to several factors. In an average year, irrigated wheat occupies less than 50% of the area but contributes up to 70-80% of the total production (Waziri et al., 2013). This unusual reliance of Afghan wheat production makes irrigated wheat crop more important from the point of food security in Afghanistan. Several new wheat varieties have been released during last decade in the country, the last one being Chonte#1 in 2010. Out of about 12 irrigated wheat varieties under certified seed production, only about eight are under breeder seed production.

Winter wheat with their higher yield potential has also been contributing to wheat production in the country. Currently country has few winter wheat varieties viz., Solh 02, Gul 96, Pamir 94 etc. These varieties have had up to 20% share in country’s certified seed production programme. Lately, varieties like Pamir 94 and Gul 96 have been showing susceptibility to yellow rust (Ahmadzada et. al., 2013) and therefore winter wheat share in certified seed has come down to about 10%.

Materials and Methods
This article reports development of a new winter wheat variety Bamyan 013, which was introduced in country during 2006-7 from Turkey-CIMMYT-ICARDA (TCI) partnered winter wheat program, Turkey. The variety being proposed was introduced at the serial no. 278 in winter wheat observation nursery with the parentage of “VORON/KUZ/4/URES/BBL//KAUZ/3/BCN”. This genotype was then tested at several high altitude locations in the country viz., Bamyan (2550 m amsl), Kabul (1841 m amsl) and Herat.
(927 m amsl) (Table 1) for four years. Number of test entries varied from 20 in first, second and fourth year testing whereas the third year trial comprised of 15 test entries. The popular national and international winter wheat varieties viz., Solh 02, Bezostaya and Katia were used as check varieties to compare the candidate variety.

Yield evaluation trials were laid out in completely randomized block design (CRBD) with three to four replications. Recommended agronomic practices were adopted to raise a good crop. Data were recorded on days to 50% flowering, days to maturity grain yield (kg/ha) and plant height (cm) in most trials during early evaluation trials. During the final evaluation trials other ancillary traits like 1000 kernel weight, grain color and other grain features described elsewhere in the paper were also recorded.

Table 1. List of ARIA Research Stations in Afghanistan where the candidate variety was tested

<table>
<thead>
<tr>
<th>No</th>
<th>Site</th>
<th>Altitude (m)</th>
<th>Mean Temperature (°C)</th>
<th>Maximum</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bamyan</td>
<td>2550</td>
<td>17.8</td>
<td>-7.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Herat</td>
<td>927</td>
<td>28.9</td>
<td>-0.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Kabul</td>
<td>1841</td>
<td>26.5</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Results

First year (2007-08): In the first year variety was tested at Bamyan in preliminary winter wheat yield trial (PWWYT). The trial had 20 genotypes including the check varieties Solh 02 and Bezostaya (Table 2). The average yield of trial was 9204 kg/ha and yield of various entries ranged from 10233 kg/ha to 7417 kg/ha. The candidate variety yielded 9233 kg/ha against 8833 kg/ha of Solh 02 and 7417 of Bezostaya. The candidate variety was as tall as Solh 02 at 97 cm. It matured in 206 days and attained 50% flowering in 155 days as against 207 and 154 days of Solh 02, respectively.

Second year (2008-09): In the second year variety was tested in the trial Advance winter wheat yield trial (AWWYT) at Bamyan (Table 2). The trial comprised of 20 entries including checks. The trial had an average yield of 4630 kg/ha and entries’ yield ranged from 5343 kg/ha to 3568 kg/ha. The candidate variety yielded 5343 kg/ha compared to 4724 of Solh 02 and 3663 of Bezostaya. The candidate matured in 270 days compared with 267 of Bezostaya and Solh 02.

Third year (2009-10): The year 2009-10 saw this variety tested at Bamyan in National winter wheat yield trial (NWWYT). This trial comprised of 15 entries (Table 2). The trial had an average yield of 6579 kg/ha and yield of entries varied from 8182 to 5259 kg/ha. The candidate variety yielded 7364 kg/ha against the 6575 kg/ha and 6658 kg/ha of check varieties. The candidate matured in 258 days and attained a height of 90 cm.

Fourth Year (2010-11): The candidate variety was tested at two locations of Kabul and Herat in final year. The NWWYT of 2010-11 had 20 entries including Solh 02 and Katia as check varieties. The average yield of trial at Kabul was 4089 kg/ha whereas at Herat, the trial mean yield was 4979 kg/ha. The candidate variety yielded an average of 4600 kg/ha across two locations compared to 4388 and 4759 kg/ha of Solh 02 and Katia (Table 2).

Discussion

Winter wheat is regarded to have an inherent higher yield potential as compared to spring wheat varieties. Traditionally Afghanistan has been growing winter wheat as several of its regions are suitable for growing this wheat ecotype. However, share of winter wheat varieties in national certified

Table 2. Average performance of proposed variety compared with check varieties

<table>
<thead>
<tr>
<th>Entry</th>
<th>2007-2008 (PWWYT)</th>
<th>2008-2009 (AWWYT)</th>
<th>2009-2010 (NWWYT)</th>
<th>2010-2011 (NWWYT)</th>
<th>Average %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg/ha %</td>
<td>kg/ha %</td>
<td>kg/ha %</td>
<td>kg/ha %</td>
<td>kg/ha</td>
</tr>
<tr>
<td>Bamyan 013</td>
<td>9233</td>
<td>5343</td>
<td>7364</td>
<td>4600</td>
<td>6635</td>
</tr>
<tr>
<td>Solh-02</td>
<td>8833</td>
<td>4.5</td>
<td>4724</td>
<td>13.1</td>
<td>6575</td>
</tr>
<tr>
<td>Bezostaya</td>
<td>7417</td>
<td>24.5</td>
<td>3663</td>
<td>45.8</td>
<td>NA</td>
</tr>
<tr>
<td>Konya</td>
<td>7617</td>
<td>21.2</td>
<td>4338</td>
<td>23.2</td>
<td>6658</td>
</tr>
</tbody>
</table>

PWWYT: preliminary winter wheat yield trial, AWWYT: Advance winter wheat yield trial, NWWYT: National winter wheat yield trial, %: % yield superiority over check
The seed production program has declined in recent past owing to lack of suitable disease resistant winter wheat varieties. This candidate variety was therefore proposed for release as a commercial variety for production by farmers.

**Yielding Potential:** WON no. 278 was tested at Bamyan, Kabul and Herat, which among themselves represent winter wheat regions of the country and this variety successfully out-yielded all the check varieties by margins ranging from 4.5% to 45% in individual years and by margins ranging from 8.2% to 31% over four year averages. The candidate variety has shown a very high level of yield stability over locations and over years as it consistently out yielded the checks. The variety was also tested in 15th Facultative and winter wheat observation nursery and yielded 6422 kg/ha across eight countries, and out-yielded Bezostaya by 17%.

**Ancillary traits:** The agronomic traits of the variety are in line with those adapted to Afghanistan as it compares very well with the most popular winter wheat variety Solh 02 (Table 3). It matures four days earlier than Solh 02 and is one cm taller than Solh 02. Its thousand kernel weight is also higher than Solh 02 by three grams and is resistant to yellow rust under Afghan conditions. The variety has been found resistant to Ug99 at Kenya during 2007-08 testing as part of 15th FAWON at # 35.

**Conclusion**
The candidate variety WON no. 278 introduced in Afghanistan during 2006-07 has shown highly stable yielding ability over four years of testing at

### Table 3. Ancillary traits of Bamyan 013 as compared with other winter wheat varieties

<table>
<thead>
<tr>
<th>Entry</th>
<th>Days to Heading</th>
<th>Days to Maturity</th>
<th>Height (cm)</th>
<th>TKW (g)</th>
<th>Highest Yellow rust score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bamyan 013</td>
<td>164</td>
<td>211</td>
<td>93</td>
<td>50</td>
<td>MR</td>
</tr>
<tr>
<td>Solh-02</td>
<td>163</td>
<td>215</td>
<td>92</td>
<td>47</td>
<td>M</td>
</tr>
<tr>
<td>Bezostaya</td>
<td>183</td>
<td>241</td>
<td>125</td>
<td>51</td>
<td>M</td>
</tr>
</tbody>
</table>

Table 4. Description of new variety Bamyan 013

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop</td>
<td>Wheat (<em>Triticum aestivum</em> L.)</td>
</tr>
<tr>
<td>Variety</td>
<td>10th WON-IR no. 278</td>
</tr>
<tr>
<td>Proposed Name</td>
<td>Bamyan 013</td>
</tr>
<tr>
<td>Type</td>
<td>Winter Wheat</td>
</tr>
<tr>
<td>Source</td>
<td>10th Winter Wheat Observation Nursery</td>
</tr>
<tr>
<td>Parentage</td>
<td>VORONA/KAUZ/4/URES/BBL/KAUZ/3/BCN</td>
</tr>
<tr>
<td>Breeding Method</td>
<td>Modified Bulk Pedigree</td>
</tr>
<tr>
<td>Developed by</td>
<td>Turkey-CIMMYT-ICARDA (TCI) Winter Wheat Improvement Programme, Ankara, Turkey</td>
</tr>
<tr>
<td>Initial Seed Source</td>
<td>CIMMYT</td>
</tr>
<tr>
<td>Proposed by</td>
<td>ARIA</td>
</tr>
<tr>
<td>Average Yield (kg/ha)</td>
<td>6635</td>
</tr>
<tr>
<td>Potential (kg/ha)</td>
<td>9233</td>
</tr>
<tr>
<td>Yield comparison with prevalent varieties</td>
<td>8.2% higher than Solh-02 in four years</td>
</tr>
<tr>
<td></td>
<td>31% higher than Bezostaya in two years</td>
</tr>
<tr>
<td></td>
<td>17% higher over multiple checks in four years</td>
</tr>
<tr>
<td>Disease Response</td>
<td>Resistant to all the prevalent diseases including Ug99</td>
</tr>
</tbody>
</table>
three winter wheat locations by yielding eight to 31% higher than various checks. Table 4 summarizes salient features of this variety. Bamyan 013 is also resistant to important diseases including Ug99 and was therefore released by Ministry of Agriculture, Irrigation & Livestock (MAIL) of the Government of Afghanistan for cultivation by farmers during August 2013.

References
The Eighth Triticeae Meeting of Japan, 2013

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The Eighth Triticeae Meeting of Japan was held at Graduate School of Agricultural Science, Kobe University on December 6 and 7, 2013. In total, 106 attendee including researchers and students participated in this meeting (Fig. 1). Recent rapid advances in life science gave us a huge amount of available information. In this meeting with 12 oral and 44 poster presentations, we considered our standing positions and discussed our further researches using wheat and barley in plant breeding and genetics fields. Especially, Dr. Matsuoka reported the genetic basis of allopolyploid evolution in wheat based on his two excellent papers recently published in PLoS ONE and International Review of Cell and Molecular Biology. The following two posters were selected as excellent works by all participants (Fig. 2 and Fig. 3), and the best poster award was given to them; “Expression alteration of class B MADS-box gene among homoeologous genes in polyploid wheat” (P41) by M. Tanaka, H. Tanaka, S. Takumi and K. Murai, and “Genotyping and structure analysis of the NBRP-Wheat tetraploid wheat core-collection” (P36) by S. Takenaka, M. Nitta, T. Kawahara and S. Nasuda. The abstracts of oral presentations and poster titles are shown below. The next meeting will be held in NARO Kyushu Okinawa Agricultural Research Center next year. I thank all participants for their joining the meeting in Kobe University.

ABSTRACTS & TITLES

Oral Presentation

O01. Effect of Tamyb10 on grain color and seed dormancy, and its utilization for wheat breeding

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Seed color is an important trait affecting flour yield and quality in wheat. Recently, it was reported that the Tamyb10 gene activated the flavonoid biosynthesis genes in wheat cultivars with the dominant allele of a red grain color gene (R). The R gene dose affects to deepen intensity of pigmentation with increasing copy number of dominant R alleles. Tamyb10 showed the similar dosage effect on grain color as the R gene genotypes. In Japanese wheat cultivars, the frequency of Tamyb10-A1b allele (functional, dominant) was 86.6%. Comparing the allele frequencies of Tamyb10-B1b and Tamyb10-D1b (61.4% and 64.6%) with those of Tamyb-A1b, the A-genome dominant allele, Tamyb10-A1b, appears to contribute more predominantly to grain color in Japanese wheat cultivars. Although each of three Tamyb10 homoeologous genes could not seem to affect germination index, wheat cultivars with two or three dominant alleles of Tamyb10 showed lower germination index than those with no or one copy of the Tamyb10 dominant allele. The dose of dominant alleles (increasing number of the functional Tamyb10 allele) affects to deepen intensity of pigmentation, suggesting that the dominant allele of Tamyb10 is related with germination inhibition through flavonoid compounds.
**O02. Beer proteome analysis and malting barley breeding**

**Takashi Iimure**
Bioresources Research and Development Department, Sapporo Breweries Ltd., 37-1, Nittakizaki, Ota, Gunma 370-0393, Japan.

Malting barley cultivar significantly affects beer quality traits such as foam stability and haze formation. Therefore, malting barley breeding in addition to malting and brewing controls should improve beer quality. Barley protein is one of the important factors in beer foam stability. Proteome analysis of beer is a powerful method to identify proteins related to beer quality traits. In our study, we constructed beer and wort proteome maps composed of two-dimensional gel electrophoresis (2DE) images and identified protein data, which is a platform for beer proteome analysis. Based on the maps, we identified two proteins, barley dimeric alpha-amylase inhibitor-1 (BDAI-1) from barley and thioredoxin (y-TRX2) from yeast as beer foam positive and negative proteins, respectively. In addition, we investigated the relationships between beer foam stability and other beer proteins such as avenin-like protein-a (ALP), B-hordein, protein Z4, and protein Z7. For protein Z4, protein Z7 and B-hordein, we developed efficient DNA markers for marker-assisted selection for better beer foam stability. We currently apply these results to our malting barley breeding program.

**O03. Toward forming an international research group for wheat quality**

**Tatsuya M. Ikeda**
NARO, Western Region Agricultural Research Center, Fukuyama, Japan

Although wheat genome sequence data has been increasing exponentially in recent years, these data is not directly contribute to understand wheat quality. For example, allelic nomenclature system for gluten proteins (glutenin subunits and gliadins), is a major determinant of differences in dough viscoelastic properties, has not been consistent in different laboratories. It has interfered with information exchange between research groups and the application of genome sequence data to understand gluten protein function. To solve these problems, it is important to share useful materials including standard cultivars to define better the relationship between proteins and processing quality attributes. Therefore, I propose a new system to share materials through public gene banks in collaboration with the Catalogue of Gene Symbols for Wheat. I also propose to form an international research group for further collaboration by forming an Expert Working Group for wheat quality under the Wheat Initiative.

**O04. Genome and transcriptome analyses of seed storage protein genes in common wheat**

**Kanako Kawaura**
Kihara Institute for Biological Research, Yokohama City University, Japan

Wheat seed storage proteins are generally classified into gliadin and glutenin. Gliadins are further separated into α/β, γ and δ, and glutenins are classified as high-molecular glutenin subunits (HMW-GS) and low-molecular glutenin subunits.
Wheat flour color is a primary indicator for estimating end use quality, thus flour color represents an important trait for selection in Japanese wheat breeding programs. Here, we evaluated flour color values using the CIE L*, a*, b* color system, with L* representing the brightness value, and a* and b* values indicating the degree of redness and yellowness, respectively. Field trials conducted under nine environments (three cropping seasons and three locations) demonstrated that genotypic variation for flour color was much higher than environmental variation. Flour protein content (FPC) showed a negative and positive correlation with L* and a* values, respectively, indicating that FPC should be taken into account when evaluating these values. To identify quantitative trait loci (QTLs) associated with flour color, association mapping was performed using 65 breeding lines from the Hokkaido prefecture of Japan. Genotypes of accessions were determined using 3,748 non-redundant markers, including SSR, DArT, SNP and gene-derived diagnostic markers. Using several statistical models corrected for population structure and/or familial relatedness, 11, 18, 13 and 2 QTLs were identified for L*, a*, b* values and FPC, respectively. Not unexpectedly, these results suggested that flour color values are controlled by a number of genetic factors, therefore effective breeding tools will be required to incorporate the superior alleles linked to the identified QTLs into target lines. Genomic selection seems to be a promising tool for this purpose, and potential approaches for the use of this methodology in improving flour color are discussed.

**O06. Molecular genetics of heading time regulation in wheat and barley and its impact on winter cereal breeding**

Kenji Kato and Hidetaka Nishida  
Laboratory of Plant Genetics & Breeding, Graduate School of Environmental and Life Science, Okayama University, Japan

Heading time is an important trait to achieve the maximum and stable production of crops in each area. Yan et al. (2003) first succeeded to identify vernalization response gene Vrn-1, and thereafter a total of nine genes controlling heading time have been successfully identified in the last decade. In this presentation, we reviewed the current status of molecular genetics of heading time regulation and summarized our findings on HvPhyC and Ppd-1. HvPhyC was identified as a
key factor to control long-day flowering in barley, through the analysis of Hayakiso-2 NILs. Hayakiso-2 carries a mutant allele of HvPhyC which causes early heading by 7-10 days in the field. The diversity analysis revealed that this mutant allele is common in the Japanese early heading cultivars of six-lowered and two-lowered barley, clearly indicating its importance in barley breeding. The second topic was focused on Ppd-1 of wheat. The photoperiod-insensitive alleles Ppd-A1a and Ppd-B1a were first found by our analysis, using DH populations derived from the Japanese wheat crosses. Ppd-A1a is often found in Hokkaido and USA, while Ppd-B1a is common in the Japanese early heading cultivars. The impact of these new alleles on winter cereal breeding was discussed.

O07. Comparative analysis of photosynthetic activity between spring wheat and winter wheat

Daisuke Takagi and Chikahiro Miyake
Laboratory of Plant Nutrition, Graduate School of Agricultural Science, Kobe University, Japan

Photosynthesis is essential for living in higher plants. In the field condition, many environmental factors that affect photosynthesis, such as light intensity and temperature changes. So it is necessary for plants to regulate photosynthesis to environmental changes. It was reported that spring wheat and winter wheat have different characteristics in photosynthesis. But many study focused on sink activity like a Calvin Benson Cycle, not the regulation of photosynthetic electron transport reaction. So it was unclear whether the photosynthetic regulation mechanisms between spring wheat and winter wheat are also different or not. In present study, we analyzed photosynthetic activity both of Chinese Spring (CS) and Mironovskaya 808 (M808) during the fluctuating light condition. It was found that quantum yield of PSII (QY (PSII)) gradually decreased and plastoquinone (PQ) became highly reduced state in CS during the analysis. On the other hand, QY (PSII) and PQ redox state were maintained in M808 during the analysis. Furthermore it was revealed that photosystem I (PSI) was severely damaged in CS but not in M808. These results indicate that PSI in CS was specifically inhibited during the analysis. Previous study indicates that PSI is specifically damaged by reactive oxygen species (ROS). So it was considered that the specific inhibition in CS derived from ROS. To make clear the effect of ROS, we conducted same analysis under low oxygen condition which ROS production in thylakoid membrane is suppressed. In this condition, the suppression of PSI activity was not detected both in CS and M808. Form these results, we found that tolerance or scavenging ability toward ROS is different between CS and M808. In this presentation, we introduce the importance of physiological analysis for future wheat breeding.

O08. Revisiting the origin of bread wheat from the viewpoint of hybrid genetics

Yoshihiro Matsuoka
Department of Bioscience, Fukui Prefectural University, Japan

Bread wheat (Triticum aestivum L. ssp. aestivum) is an allohexaploid species (genome constitution AABBDD) that formed through a natural hybrid cross between a cultivated form of tetraploid Triticum wheat (T. turgidum L., genome constitution AABB, the female parent) and a wild diploid species Aegilops tauschii Coss. (genome constitution DD, the male parent). The hybrid cross produces triploid F1 hybrids (genome constitution ABD) that spontaneously undergo genome doubling and set hexaploid F2 seeds. This scenario for the origin of bread wheat is widely accepted but the details of its early episode are yet to be clarified. My talk first provides an overview of field studies that addressed the questions about how the T. turgidum-Ae. tauschii hybrid cross took place in a natural setting, and then focuses on recent findings on the genetic basis for the genome doubling of triploid F1 hybrids. I discuss the implications of the findings for the evolution of bread wheat.

O09. How shall we study molecular mechanisms of the gametocidal function?

Shuhei Nasuda
Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University, Japan

Gametocidal (Gc) chromosomes are selfish alien chromosomes introduced into wheat which ensure preferential transmission by killing gametes without themselves. In this talk, I reviewed the previous studies on the mode of action of the gametocidal chromosomes. The knowledge accumulated so far is mostly based on cytogenetic analysis of the gametogenesis, which tells us that
the main cause of sterility is the chromosome breakage in the gametes lacking the Gc factor. The breakage occurs in the pollen under post-meiotic mitosis. Analysis of a mutant revealed that (1) Gc action is under control of a gene, (2) Gc action is composed of two functions; protecting and breaking functions. These two functions can be genetically dissected because the mutant that lacks only the breaking-function. We are now trying to clone the Gc gene for various approaches. The scheme of gene cloning and associated studies is discussed.

O10. Self-incompatibility in a wild relative of barley, *Hordeum bulbosum*

Katsuyuki Kakeda
Graduate School of Biosources, Mie University, Japan

Self-incompatibility (SI) in the grasses (Poaceae) is gametophytically controlled by a distinct two-locus genetic system governed by the multiallelic loci $S$ and $Z$. In this system, the incompatible reaction occurs only when both the $S$ and $Z$ alleles in the pollen grain match those in the pistil. Thus, the phenotype of SI is thought to be determined by a complementary interaction of the $S$ and $Z$ genes. I have employed diploid *Hordeum bulbosum*, a wild relative of cultivated barley (*Hordeum vulgare*), for studying the molecular mechanisms of the two-locus SI system. From recent studies of my research group, a pistil-specific clone, named HPS10, has been identified as a good candidate of the $S$ gene on the female side. This gene displays complete linkage to the $S$ locus, has a high degree of allelic sequence polymorphisms, and encodes a putative ligand-like molecule. Further characterization and in vitro bioassay of the HPS10 protein were presented.

O11. Comparative mitochondrial genomics of *Triticum* and *Aegilops* using next-generation sequencing

Mai Tsujimura$^1$ and Toru Terachi$^{1,2}$

$^1$Plant Organelle Genomics Research Center, Kyoto Sangyo University, Japan
$^2$Faculty of Life Sciences, Kyoto Sangyo University, Japan

Comparing to the mammalian mitochondrial genomes, plant mitochondrial genomes have complex structure and exhibit unique properties. For example, the genome size is large and varies among species, ranging from ca. 200 kb to 11,000 kb, and the “mitochondrial genome” is believed to be an entity that is present as multipartite circular molecules due to active homologous recombination between repeated sequences in a master molecule. In order to reveal phylogenetic relationships among cytoplasm donors to polyploid wheat, we determined the complete mitochondrial genome sequences of alloplasmic lines of common wheat with *Triticum timopheevi* and *Aegilops speltoides* cytoplasms using next-generation sequencing. Contigs consisting of many reads were assembled into the master molecules of 443,419 bp and 476,091 bp for *T. timopheevi* and *Ae. speltoides*, respectively, and 55 genes including rRNA and tRNA were identified in each genome through the comparative annotation with the sequence of *Triticum aestivum* (DDBJ: AP008982). The homologies among syntenic regions in three mitochondrial genomes are quite high (more than 90%), but the syntenic regions scattered differently in each genome, suggesting that numerous rearrangements had occurred among three mitochondrial genomes during evolution of the species.

O12. Epigenetic regulation of a flowering promoter gene VRN1 controlled by mitochondrial genome

Koji Murai
Department of Bioscience, Fukui Prefectural University, Japan

Cytoplasmic substitution (alloplasmic) lines of wheat (*Triticum aestivum*) that carry the cytoplasm (mitochondrial genome) of the wild relative *Aegilops geniculata* show delayed flowering-time. *VRN1* encoding APETALA1/FRUITFULL-like MADS box transcription factor plays a central role in the flowering pathway. In the euplasmic lines, the expression level of *VRN1* reached to a threshold level at the 3-4 leaf stage under long day conditions after vernalization, accelerating flowering. On the other hand, the alloplasmic lines showed the lower expression level of *VRN1*, and the expression reached to the threshold at latter stage. It has been known that *VRN1* expression is epigenetically suppressed before vernalization, and is upregulated after vernalization by epigenetic alteration. Bisulfate sequencing analysis and ChIP analysis with H3K27me3 antibody revealed that epigenetically repressive state is maintained in *VRN1* locus of
the alloplasmic line after vernalization. These suggest that mitochondrial genome affects the epigenetic state of nuclear VRN1 gene through a mitochondrial retrograde signaling. Using comparative RNA-seq analysis in the seedlings of alloplasmic and euplasmic lines, we detected four candidate mitochondrial genes associated with the epigenetic state in VRN1. They all are chimera genes encoding a novel ORF.

Poster Presentation

**P01.**
Toward cloning of *PBY4*, an avirulence gene of *Setaria* isolates of *Pyricularia oryzae* against barley

**P02.**
Expression profiling of *Fusarium graminearum* toxin related genes in wheat spikes
Harada, M. and T. Ban (KIBR, Yokohama City Univ.)

**P03.**
Molecular pathogenicity of wheat-*Fusarium graminearum* interaction
Kosaka, A., M. Alagu, D. Kajihara and T. Ban (KIBR, Yokohama City Univ.)

**P04.**
Characterization and enhancement of Afghan wheat landraces for strip rust
Stanikzai, A.S., M. Alagu and T. Ban (KIBR, Yokohama City Univ.)

**P05.**
Effects of acetic acid on drought tolerance in wheat
Takeda, H. (KIBR, Yokohama City Univ.)

**P06.**
Does a cold-responsive WLT10 protein have ability of extracellular movement?

**P07.**
Sowing depth and drought study on Afghan wheat Landraces
Osmani, A.A., M. Alagu and T. Ban (KIBR, Yokohama City Univ.)

**P08.**
Studies of Afghan wheat landrace under osmotic stress
Kanno, M., M. Alagu and T. Ban (KIBR, Yokohama City Univ.)

**P09.**
Comparative analysis of photosynthetic activity between spring wheat and winter wheat

**P10.**
The endogenous hormonal levels of the calli derived from different organs in barley
Hisano, H., T. Matsuura, I. Mori, M. Yamane and K. Sato (Institute of Plant Science and Resources, Okayama Univ.)

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**Fig. 2.** The 8th Triticeae Meeting of Japan, 2013. Poster presentation.
P11. Efficient transformation method for bread wheat mediated with Agrobacterium
Kamiya, H.¹, Y. Ishida² and M. Isshiki¹ (¹KIBR, Yokohama City Univ., ²Plant innovation center, Japan tobacco Inc.)

P12. Functional analysis of wheat HMGR genes involved in the sterol biosynthesis
Hamada, S. (KIBR, Yokohama City Univ.)

P13. Gene cloning of wheat WRKY transcription factor genes activated in hybrid necrosis

P14. Alleviation of necrotic symptom by ZnCl₂ treatment in wheat hybrid necrosis

P15. Expression analysis of flowering-time genes in extra early-flowering mutants during vegetative growth phase

P16. Identification of novel mitochondrial genes associated with the epigenetic regulation of a flowering promoter gene VRNI
Ozeki, M.¹, S. Kitagawa¹, Y. P. Gyawali², T. Terachi² and K. Murai¹ (¹Dep. Biosci., Fukui Pref. Univ., ²Fac. Life Sci., Kyoto Sangyo Univ.)


P18. Expression analysis of phytochrome genes on bulb formation in wild barley Hordeum bulbosum L.
Kobayashi, Y., M. Alagu and T. Ban (KIBR, Yokohama City Univ.)

P19. Search for the female and male S factors involved in the self-incompatibility of Hordeum bulbosum
Hashimoto, S., R. Asahara, T. Hiramatsu and K. Kakeda (Grad. Sch. Bioresources, Mie Univ.)

P20. Evaluation of evolutionary stability of chondriome using alloplasmic lines of common wheat
Yotsumoto, T.¹, N. Mori¹ and K. Tsunewaki² (¹Lab. Plant Genetics, Grad. Sch. Agric. Sci., Kobe Univ., ²Prof. emeritus, Kyoto Univ.)

P21. Molecular structure of novel high-molecular-weight glutenin subunit genes associated with strong dough in 1E chromosome of Thinopyrum elongatum
Fig. 3. The 8th Triticeae Meeting of Japan, 2013. Poster presentation.

P22. Chromosome assignment of gliadins in common wheat
Miura, M. (KIBR, Yokohama City Univ.)

P23. Functional analysis of transcription factors regulating seed storage proteins using the RNAi method in common wheat
Kouyama, H. (KIBR, Yokohama City Univ.)

P24. Screening of parent material for breeding barley varieties containing high level of β-glucan in grain
Tonooka, T., N. Kawada, H. Araki, T. Yanagisawa (NARO Kyushu Okinawa Agricultural Research Center, NARO Institute of Crop Science)

P25. Seed dormancy in wild wheat: Phenomena and a new insight into its change during early domestication process

P26. Genetic control of seed dormancy in wild emmer wheat
Kakoya, S., N. Uenomachi, R. Kariyasu and S. Ohta (Dep. Bioscience, Fukui Pref. Univ.)

P27. Diversity in seed dormancy and the morphological characters correlated with it in natural populations of wild diploid wheat

P28. Diversity in seed dormancy and the morphological characters correlated with it in natural populations of wild tetraploid wheat

P29. Genetic analysis of domestication traits in emmer wheat

P30. Genetic diversity and differentiation of chloroplast genome within and among natural populations in wild emmer wheat

P31. Study of Afghan wheat Landraces to identify new ediotypes for high yielding potential
Maqsodi, A.M., M. Alagu and T. Ban (KIBR, Yokohama City Univ.)

P32. Large scale phenotyping of barley germplasm using "FieldBook"

P33. Development of a high-throughput DNA extraction system from a large number of wheat seedlings

P34. Variation for ABA responsiveness in the core collection of hexaploid wheat in National Bioresource Project

P35. Variation for awn length in the core collection of hexaploid wheat in National Bioresource Project

P36. Genotyping and structure analysis of the NBRP-Wheat tetraploid wheat core-collection
Takenaka, S., M. Nitta, T. Kawahara and S. Nasuda (Grad. Sch. Agri., Kyoto Univ.)

P37. Characterization of novel spike compactness genes using Chinese Spring EMS-mutants
Uchiyama, H. (KIBR, Yokohama City Univ.)

P38. Genetic mapping of a non-glaucousness gene Iv2 in wheat

P39. Genetic mapping of a low temperature-induced hybrid necrosis gene Net2 in synthetic wheat

P40. Cytologically based physical maps of chromosome 1R derived from two different rye species
Li, J., Y.P. Gyawali, S. Nasuda and T.R. Endo (Lab. Plant Genet., Grad. Sch. Agri., Kyoto Univ.)

P41. Expression alteration of class B MADS-box gene among homoeologous genes in polyploid wheat

P42. Transcriptome changes during allopolyploidy and genome stabilization in wheat
Jung, Y.¹, K. Kawaura¹, K. Mishina¹, S. Sakuma¹, M. Kishii² and Y. Ogihara¹ (¹KIBR, Yokohama City Univ., ²CIMMYT)

P43. Comparative analysis of the transcriptomes among common wheat and its ancestors using RNA-seq
Kajita, Y.¹, K. Mishina¹, Y. Kamiya¹, K. Kawaura¹, K. Mochida², H. Tarui¹, N. Suzuki³, J. Kawai³ and Y. Ogihara¹ (¹KIBR, Yokohama City Univ., ²RIKEN BMEP, ³RIKEN OSC)

P44. Gene expression and molecular evolution analyses of hexaploid wheat with references to the genomics and RNA-seq data sets of diploid progenitors
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Abbreviations should be explained at first occurrence.

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