Wheat Inf. Serv. 116, 2013. www.shigen.nig.ac.jp/ewis

Wheat Information Service

Electronic Newsletter for Wheat Researchers

No. 116

CONTENTS

Research Information

Research Opinion & Topics

Meeting Reports

Others

Wheat Inf. Serv. 116: 3-8, 2013. www.shigen.nig.ac.jp/ewis

Research Information

Marker Assisted Selection for Complementation of *Glu-A1b*, *Glu-B1i*, and *Glu-D1d* alleles in Early Segregating Generations of Wheat

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Abstract

Marker assisted selection (MAS) is believed to revolutionize breeding practices through improved improve efficiency and precision of selection, but the implementation of the technology is restricted to limited public funding. Here we demonstrate MAS procedure with significant reduction of workload to fix alleles in early generations. In recent years, advancements in molecular genetics resulted in identification of DNA tags associated with specific alleles of high-molecular-weight glutenin subunits (HMW-GSs) loci involved in bread making quality i.e., *Glu-A1*, *Glu-B1*, and *Glu-D1*. In this study, we report on utilization of three molecular markers selecting for alleles attributed to high gluten strength i.e., *Glu-A1b* (2*), *Glu-B1i* (17+18), and *Glu-D1d* (5+10). Field grown segregating wheat plants at F_2 generation of an intercross (with allelic complementarity) were subjected to selection by using three molecular markers. Individuals deemed positive on the three markers were selected and the corresponding seeds were used to constitute F_3 generation. Nearly 15 promising heads per each F_3 family were tagged and the corresponding flag leaves were collected for marker analysis. The heads corresponding to flag leaves that were positive for the three markers were selected to proceed with F_4 generation. Marker analysis of DNA extracted from seedlings at F_4 generation revealed that F_4 individuals selected contain all three *Glu-A1b* (2*), *Glu-B1i* (17+18), and *Glu-D1d* (5+10).

Key words: wheat, marker assisted selection, HMW-GS, allele specific marker

Introduction

Decades of investment in biotechnology has resulted in tools i.e. tissue culture, doubled haploid production, transgenic organisms, and DNA tags to revolutionize breeding science. DNA tags play as landmarks guiding the selection of chromosomal segments of interest in breeding procedures. The use of DNA tags may facilitate accumulation of multiple genes of interest in a breeding material or so-called gene pyramiding. Tracking chromosomal segments and selections thereby in breeding materials by means of molecular markers and DNA tags is referred to as marker assisted selection (MAS) (Dubcovsky et al., 2004). However, this methodology has not yet been implemented in many public wheat breeding programs (Koebner and Summers, 2003). MAS would also improve efficiency and precision of conventional plant breeding (Collard and Mackill, 2008). In MAS procedure, gene of interest is naturally transferred through meiotic chromosomal recombination and therefore, public perception is not against implementation of such technologies programs. However, implementation of such technologies is delayed due to restricted public funding (Dubcovsky et al., 2004).

Bread making quality is associated with glutenin composition and properties (Bottomley et al., 1982; Huebbner and Wall, 1976). Glutenin properties control dough resistance and extensibility(Eagles et al., 2006) and gluten elasticity and extensibility have significant influence on dough viscoelasticity (Belderok, 2000) and bread making quality of wheat (Nieto-Taladriz et al., 1994). Gluten consists of high-molecular-weight glutenin subunits (HMW-GSs) and low-molecular-weight glutenin subunits (LMW-GSs) (Lindsay et al., 2000). HMW-GSs are encoded by loci *Glu-A1*, *Glu-B1*, and *Glu-D1*, collectively referred to as known as *Glu-1* loci (Ammar et al., 2000; Payne et al., 1981; Payne et al., 1987). Allelic variations in *Glu-A1*, *Glu-B1*, and *Glu-D1* were reported to have strong association with gluten strength (Payne et al., 1981; Payne et al., 1987). Traditionally, public breeding programs evaluate pure lines produced at the end of each cycle of breeding for examination of allelic polymorphism of HMW-GS by using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) systems. However, this approach helps in detection of HMW-GS alleles rather than enabling breeders to play selecting role in pyramiding the high scoring alleles. In contrast to such approach, tracing of genes and alleles of interest in segregating populations by using molecular markers at DNA level enables breeders to precisely select for promising segregants with desired recombination. This communication reports the use of DNA tags for precise selection of segregants that possess high score alleles at *Glu-1* loci i.e., *Glu-A1b* (2*), *Glu-B1i* (17+18), and *Glu-D1d* (5+10).

Materials and Methods

In this study we demonstrated MAS in early generations (F_2 and F_3) by selecting for high scoring alleles for improved bread making quality in a public wheat breeding program. Segregating wheat materials included F_2 generation from an intercross made between cultivars and breeding materials including, Parsi, Pishtaz, M-84-14, and M-83-17 as parental lines as demonstrated in Fig. 1A. M-83-17 is a breeding line obtained from a cross between Alvand and BACANORA-88. Alvand (pedigree: CF1770/1-27-6275) is a facultative wheat released for moderate and cold regions of Iran in 1995. Because 1-27-6275 is a landrace, Alvand is known to be moderately tolerant to drought, salinity, and frost. BACANORA-88 (pedigree: JUPATECO-73/(SIB)BLUEJAY//URES-81) is a spring wheat developed and released by CIMMYT in Mexico in 1988. Parsi named after Persian language (pedigree: Dove"S" / Buc"S" // 2* Darab) is a wheat cultivar released in 2011 by wheat program Iran for moderate region. Parsi is a product of backcross conventional breeding between a maternal parent named DOBUC 1 (pedigree: Dove"S" / Buc"S") developed by CIMMYT/ICARDA in 1995 as the donor parent which shows moderate drought tolerance in Iran and a paternal recurrent germplasm from Iran named Darab. Darab (named after the local area of germplasm enhancement in Darab located at Fars province) is a relatively old red spring wheat cultivar released in 1980 for warm and dry regions of Iran. Pishtaz (equivalent to Frontier in English) is a modern spring cultivar released in 2002 for moderate region with terminal drought tolerance. Pishtaz is a progeny of a cross between Alvand and a Brazilian cultivar Aldan / Ias58. Aldan / Ias58 has shown durable rust and Karnal bunt resistance in India for over a decade. 20 M-84-14 is a breeding line obtained from a cross between Gonam (Ww33 / Vee"S") and Niknejad. Gonam is a spring wheat cultivar developed by CIMMYT / ICARDA in 1989. And Niknejad (pedigree: F13471/Crow"s") is a spring wheat cultivar developed by ICARDA and released in Iran in 1995 showing tolerance to limited water availability. The DNA diagnostic test for parental cultivars and breeding materials is given in Fig. 1B.

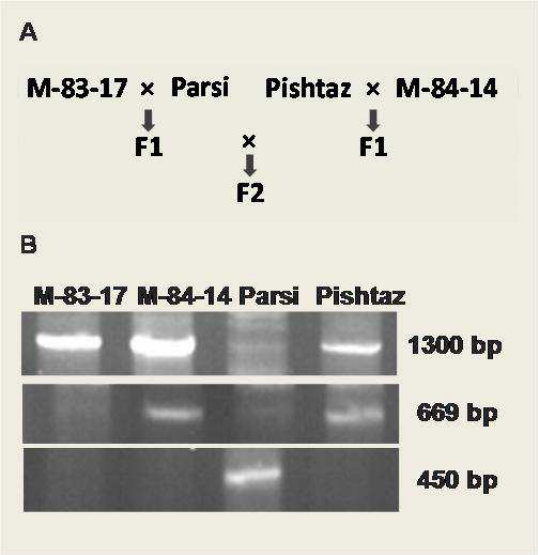

Fig. 1. A demonstrates the intercross schema made by using the parental breeding materials and B represents presence or absence of *Glu-A1b* (top), *Glu-B1i* (middle), and *Glu-D1d* (bottom) in parental lines.

Forty F_2 individuals were selected and tagged in the field from which a segment of leaf was collected. DNA was extracted from leaf tissue using a rapid and small-scale DNA isolation by flash freezing the excised leaf sample from individual F_2 segregants in liquid nitrogen followed by grinding to find powder. Extraction was then performed in 2 mL sterile tubes. A quarter of the volume of 2 mL tube was filled with frozen leaf powder and 0.9 mL of preheated (up to 65ºC) CTAB buffer [2% (w/v) cetyl trimethyl ammonium bromide, 200 mM Tris/HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl, and 1% (v/v) freshly added β-mercaptoethanol] was added. Samples were heated for 45 min at 65ºC. 900 µL Pre-cooled 24:1 (chloroform : isoamylalcohol) mixturewas added to the heated tubes. The extraction mixes were centrifuged at 12000 rpm for 15 min at 4ºC. The upper layer was then collected and transferred into a new tube and was supplemented with 700 µL of pre-cooled (-20ºC) isopropanol and 100 µL of 3 M ammonium acetate. DNA was precipitated by incubating at -20ºC for at least one hour. The precipitates were pelleted by centrifugation at more than 11000 rpm for 10 min at 4ºC. The pellets were washed with 300 µL of pre-cooled 70% (v/v) ethanol. The DNA pellets were dissolved in 70 µL of nuclease free water. MAS within F_2 and the successive F_3 generations was performed by DNA tags after a pre-selection made by breeder based on plant sand and ideotype.

For selecting for allele *Glu-A1b* (2*) and *Glu-B1i* (17+18) allele against other alleles at *Glu-A1* and *Glu-B1* loci, respectively, we have used primers reported by Ma et al. (2003). *Glu-A1b* allele was traced by using primer pairs Fwd: 5'-ATGACTAAGCGGTTGGTTCTT-3' and Rev: 5'-ACCTTGCTCCCCTTGTCTTT-3'. *Glu-B1i* allele was traced by using primer pairs Fwd: 5'- CGCAACAGCCAGGACAATT-3' and Rev: 5'-AGAGTTCTATCACTGCCTGGT-3'. For selecting for *Glu-D1d* allele at *Glu-D1* locus we have used primer pairs developed by Vjell (1998) i.e., Fwd: 5'-GCCTAGCAACCTTCACAATC-3' and Rev: 5'-GAAACCTGCTGCGGACAAG-3'. The cycling conditions were as described by Ma *et al*. (2003) and Vjell (1998). DNA templates containing *Glu-A1b*, *Glu-B1i*, and *Glu-D1d* alleles are able to yield PCR products of sizes ~1319 bp, 669 bp, and 450 bp, respectively (Ma et al., 2003; Vjell, 1998).

PCR reaction mixes of 25 µL final volume contained 1 µl DNA, 2.5 µl 10X Taq buffer, 1.5 mM $MgCl_2$, 2.5 mM of each dNTP, 0.4 pmol of each primer, 1 unit of Taq polymerase. PCR amplifications for *Glu-A1b*, *Glu-B1i*, and *Glu-D1d* alleles consisted of an initial denaturing step of 94ºC for 4 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 59ºC for 30 s, and extension at 72ºC for 90 s. The PCR reactions were terminated by a final extension of 72ºC for 10 min. PCR reactions were performed in an Eppendorf Mastercycler® (Eppendorf AG, Hamburg, Germany). For visualization, PCR products were run on 1% or 1.5% agarose gel electrophoresis followed by ethidum bromide staining. Imaging was performed by a UVItec Gel Document system (UVItec Limited, Cambridge, UK).

Results and Discussion

Segregating wheat plant individuals at F_2 generation of an intercross made from four parental breeding materials were subjected to selection twice a cropping cycle (F_2 and F_3) once through breeder based on visual inspection and afterward on the basis of molecular markers. Breeder's selection was exerted at F_2 generation resulting in 40 segregants. Thereafter, we traced HMW-GS alleles of interest by DNA tags to further screen the segregants based molecular data. Out of 40 progenies pre-selected by breeder, 15, 21, and 15 segregants demonstrated to possess *Glu-A1b*, *Glu-B1i*, and *Glu-D1d* high score HMW-GSs at *Glu-A1*, *Glu-B1*, and *Glu-D1*, respectively. Sixteen segregants were negative on all three markers tested. Seven segregants carried only one of the alleles. Four segregants were positive on two out of three markers tested. Only 12 segregants were positive on all examined markers. A representative gel picture of allele specific primers at F_2 generation is shown in Fig. 2A. Seeds from these 12 segregants were collected and were planted next cropping season each on 2 rows of 2 meters long to constitute 12 F_3 families. At F_3 generation, breeder's selection was exerted to select six families from the 12 F_3 families. Nearly 15 promising heads per each family were tagged and the corresponding flag leaves were collected for marker analysis. The heads corresponding to flag leaves that were positive for the three markers were selected to proceed with F_4 generation. A representative gel picture of PCR products for seven F_2 derived F_3 families is shown in Fig. 2B. Once selected for those F_2:F_3 individuals deemed positive for the three markers, molecular analysis of DNA extracted from seedlings of selected plants at the next generation (F_4) revealed that F_4 individuals selected contain all three *Glu-A1b* (2*), *Glu-B1i* (17+18), and *Glu-D1d* (5+10) (Fig. 2C).

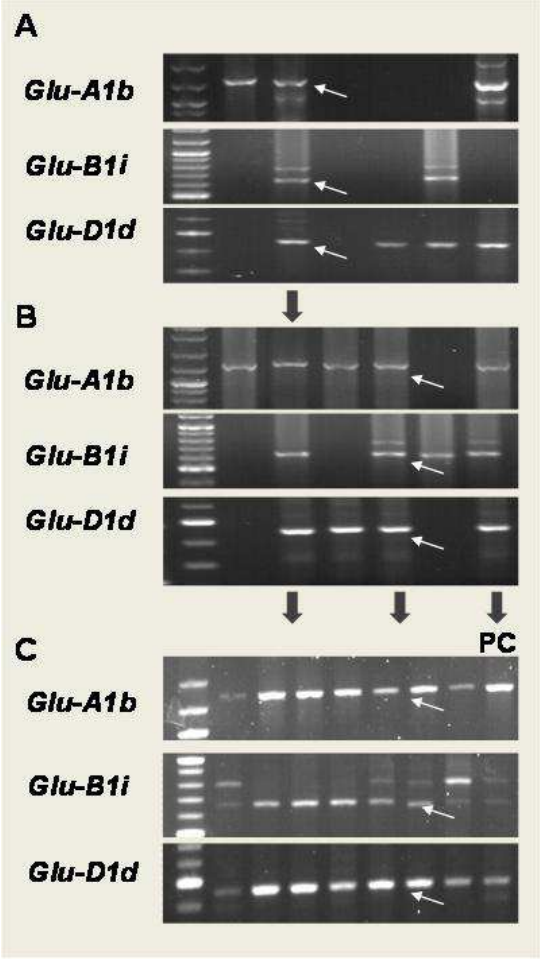

Fig. 2. A represents analysis of DNA tags from flag leaves in a selected number of F_2 individuals from which only the second (from left) F_2 segregant appeared to possess all three alleles; B represents presence / absence of the three alleles in a selected F_3 segregants; and C is the demonstration of DNA analysis in a selected seedling plants at F_4 generation indicating all F_4 individuals that were selected via MAS possess all three alleles. The lane PC represents positive control samples for *Glu-A1b* in cultivar 'Chenab'(upper figure), *Glu-B1i* in cultivar 'Golestan'(middle row figure), and *Glu-D1d* allele in cultivar 'Inia' (lower row figure), respectively.

The availability of knowledge for mechanisms underlying aspects of end-use quality traits in wheat and the wealth of sequences for various alleles of the genes involved in end-use quality enabled the design and application of diagnostic DNA markers and implications thereof in wheat breeding programs (Gale, 2005). Selection is then possible for segregants and lines with positive tests on markers without the need for the direct assessment of traits. These breakthroughs further the efficiency and speed of the development of cultivars with improved quality in the future (Gale, 2005). Several MAS procedures have been implemented so far in breeding programs. Examples include the work reported by de Bustos et al. (2001), Radovanovic and Cloutier (2003), and Kuchel et al. (2007). MAS was applied on a backcross population by de Bustos et al. (2001) to improve glutenin quality Spanish wheat. Kuchel et al. (2007) have applied molecular markers for assisted breeding coupled with doubled haploid procedure for multiple loci in a back cross population targeting both disease resistance and end-use quality. They have used DNA tags to select for Lr34/Yr18, Lr46/Yr29, and glutenin subunits all at once and at haploid stage. They have concluded that integration of MAS for specific traits at the early generations of segregation substantially increase breeding gain in wheat. Radovanovic and Cloutier (2003) have combined benefits from MAS and doubled haploid procedure for HMW-GS. In our study, a composite cross with high allelic complementarity was made for the moderate climate region of Iran and then favorable *Glu-1* alleles were captured from different parental lines at early generations i.e. F_2 and F_3 by means of DNA tags. A workflow similar to our strategy was reported by Ribaut and Bertan (1999), where the authors have fixed specific loci early in segregating generations while maintaining the rest of the genome as much segregating as possible. They named their strategy as single large-scale marker-assisted selection. In their study, they conducted MAS only once and selected plants homozygous for favorable alleles at target loci while exerting no selection pressure for genomic regions outside of the target region. Although the cost, time, and workload required for MAS procedures has been always a setback for MAS implementation, and solutions for reducing costs has been offered such as a rapid genotyping of a large number of samples coupled with direct staining of DNA with ethidium bromide without electrophoresis as reported by Gu et al. (1995), it is worth to note that MAS coupled with breeder's interference and conscious selection made by breeders results in significant reduction of workload and reduces the number of segregants to be analyzed to a great extent. Our approach was a combination of conventional breeding and MAS where at F_2 generations we selected 40 individuals with our breeding experience as if we were not going to applied MAS pressure. Then the DNA samples of these 40 individuals were analyzed at the laboratory to confirm presence or absence of high scoring

alleles. Individuals possessing three high score alleles at *Glu-1* loci were selected. Thus far, we have generated genetic variation satisfying conventional breeding and molecular breeding. In F_3, we have exerted another round of breeder selection and molecular marker analysis to satisfy traits that are naturally being selected from conventional perspectives and to fix the *Glu-1* genomic targets for high score alleles. Therefore, in contrast to what presented by Ribaut and Bertan (1999), our approach does not seem to require large populations, at least early in segregating generations, to achieve our goal of (haplotype) chromosomal segment engineering.

Acknowledgement
This work was financially supported by the Seed and Plant Improvement Institute, Iran under grant number 2-03-03-90018 to Dr. Mohsen Mohammadi.

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Wheat Inf. Serv. 116: 9-11, 2013. www.shigen.nig.ac.jp/ewis

Research Opinion & Topics

Strategic plan and approaches for Afghanistan wheat improvement under SATREPS

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Abstract

Rapidly growth of the global population and climate change are great concern for sustainable global food production and environment conservation in developing countries. Wheat breeding and genetic studies will find a way of great advantage for sustainable development mitigating the global threats. Particular in Afghanistan, the re-establishment of farming system is one of the major contributions to poverty reduction and social welfare after the civil war. The accumulation of scientific knowledge, technology development, researcher's training, the restoration of related facilities and effective extension services are essential to establish future generation. Wheat is the most important crop in Afghan which accounts for 77% of all cereal production (Anonymous, 2010), but its production volumes are not able to meet demand due to low productivity under the unfavorable natural condition, mainly of drought (Fig. 1). While wheat production currently depends on rain fall where covered over 75% area of cultivation, the expansion of irrigation system and wheat breeding system adapted to marginal lands in the Afghan climate will be critical and challenging (Agriculture Prospects Reports, 2012). Our new five years project 'Development of wheat breeding materials for sustainable food production in Afghanistan' launched in 2011 April. Aim of this project is development of wheat breeding system for sustainable food production in Afghanistan to conserve the local varieties and wild relatives of wheat, and maximizing their potential as a breeding material (pre-breeding) for high yield and good quality in parallel with capacity development.

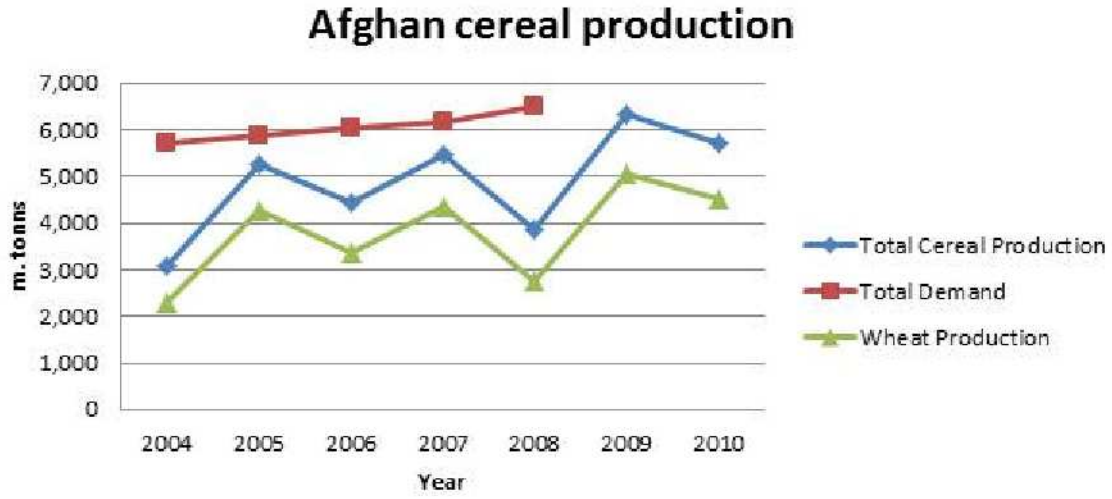

Fig. 1. Cereal production scenario in Afghanistan.

SATREPS – *Science and Technology Research Partnership for Sustainable Development*

Based on the needs of developing countries, Japan Science and Technology Agency (JST) cooperates with Japan International Cooperation Agency (JICA) to entails promotion of international joint research targeting global issues and envisaging future utilization of research outcomes with systemic reforms (http://www.jst.go.jp/global/english/). Implemented through collaboration with Official Development Assistance (ODA), the aim of the program is to acquire new knowledge leading to resolution of global issues and advancement of science and technology. Such international joint research under the program will also address the research and development of capacity and contribute to the sustained research activities in developing countries.

Framework and activities of project

This project intends to discover and conserve useful wheat germplasm with highly adapted to marginal lands. The useful experimental lines will be used for cross breeding of superior wheat variety to develop new breeding materials combining drought tolerance, disease resistance, high yielding and high quality traits. Kihara Institute for Biological Research, Yokohama City University have conserved valuable wheat germplasm, including around 500 Afghan wheat local varieties and wild relatives, which Professor Hitoshi Kihara and team explored and collected from 1950's to 1970's in Afghanistan by Kyoto University Scientific Expedition. This project conducts research for development on the genetic diversity and agricultural characteristics of these wheat collections through international collaboration. The collections are expected to be brought back to Afghanistan along with the well-trained Afghan researchers. The project targets to develop novel breeding materials for future Afghan wheat breeding system with adapted to marginal lands. It would be helpful and valuable to reconstruct the wheat breeding system for sustainable crop production in Afghanistan, which can contribute as a core function to increase wheat production in Afghanistan. Ministry of Agriculture, Irrigation and Livestock (MAIL), Afghanistan is our main counterpart with whom we made the pipeline to achieve our project ideas. Further the project is catalysts by Japanese experts (including Tottori University and RIKEN PSC) and international networks through CGIAR Centers (CIMMYT and ICARDA) contribute in all aspects of project. The overall framework of our project is showed in Fig. 2.

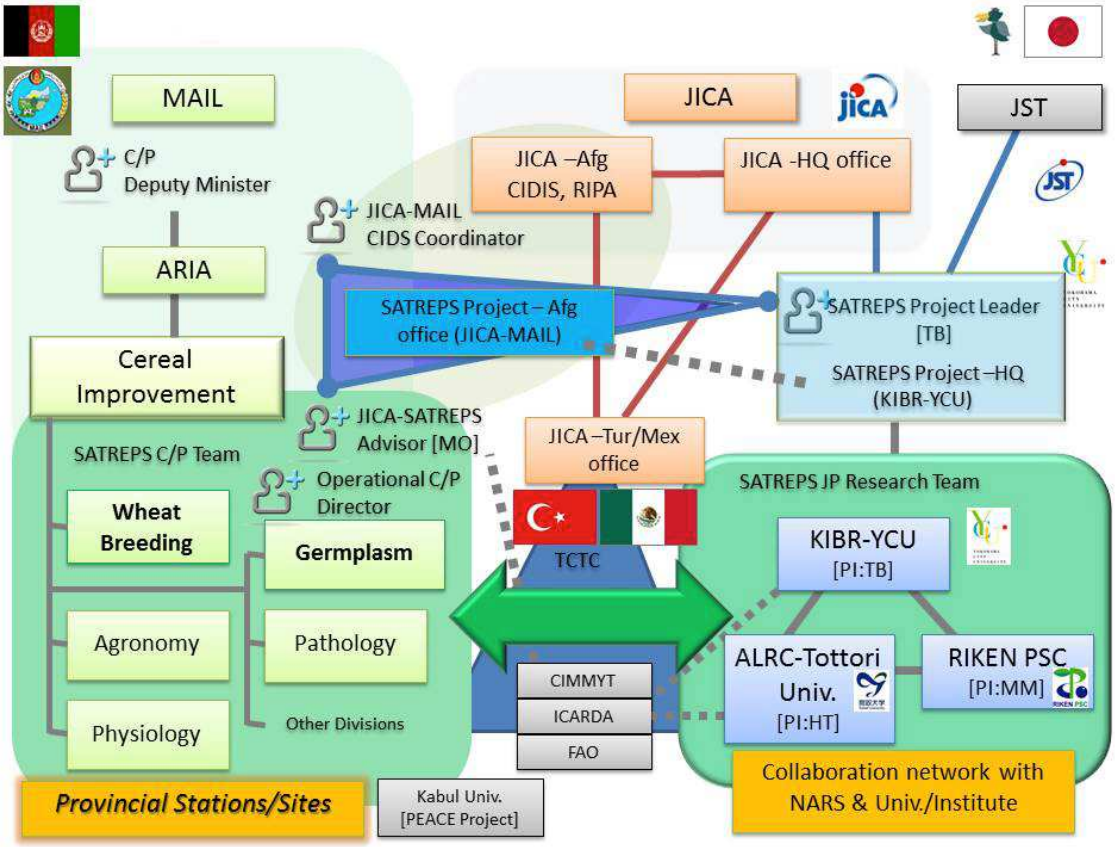

Fig. 2. SATREPS project operational structure.

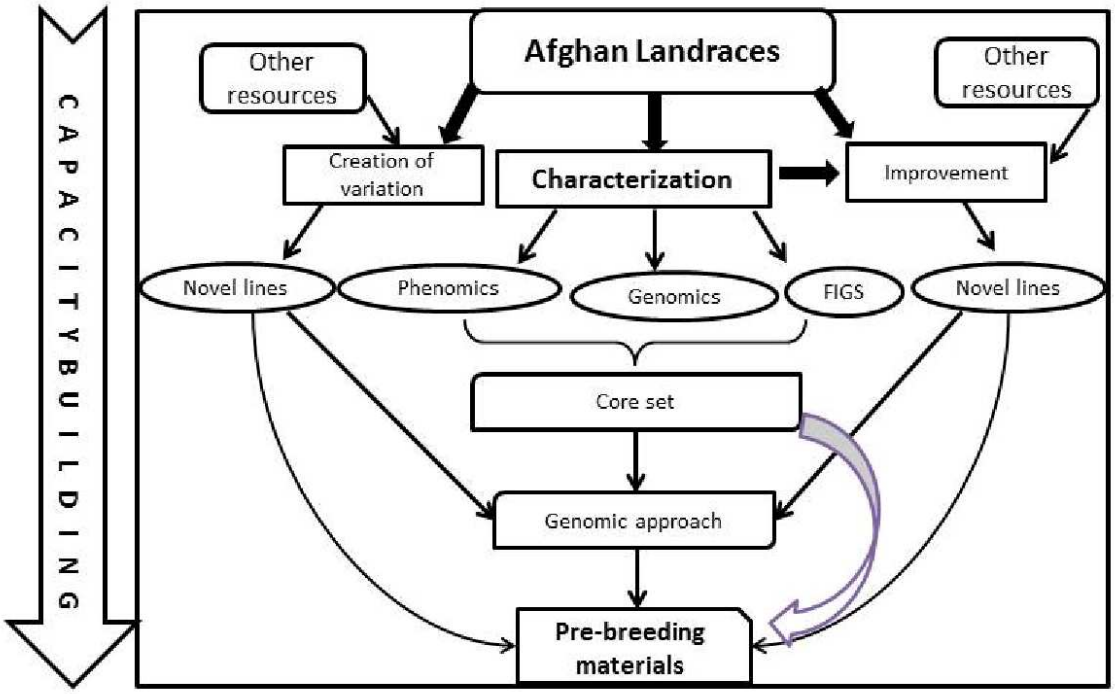

Fig. 3. Wheat germplasm enhancement and tapping genetic potential.

Activities and expected outcomes of the project
By keeping the target of future Afghanistan wheat improvement in mind, the following targets are outlined.

a) Assessment of genetic diversity for Afghan wheat landraces for rain-fed wheat improvement
b) Development of practical wheat improvement system in MAIL with capacity development of Afghan researchers
c) Development of novel wheat breeding materials with widely adaptation introduced from the wheat landraces and wild relatives are developed
d) Conservation and utilization of Afghan wheat germplasms

The scientific outline or approaches for the projects is showed in Fig. 3. The expected outcomes from this project in five years (2011-16) are

1. Accumulation of knowledge on the genetic diversity among wheat germplasm originated from Afghanistan to improve adapted commercial wheat varieties;
2. Development of practical breeding and evaluation methods for wheat germplasm highly adapted to marginal lands in Afghanistan;
3. Development of novel wheat breeding materials with widely adaptation introduced from wheat wild relatives; and
4. Afghan wheat germplasm are conserved and utilized
5. Short term, long term training (Master courses) and technical workshops for capacity development.

Current status
Based on clear goals and plan, the project made the good foundation for the research and capacity development. Through counter-part, national and international collaboration, the phenotypic characterization of Afghan landraces for various morphological, elemental composition and biotic stresses were started last year which has been continuing in the consecutive years to get concise data. Molecular characterization is underway with high-through put markers in order to get the genome wide data for diversity and population structure analysis. Based on phenomics and genomics data, establishing Afghan wheat core-set is possible to carry-out further in-depth research and application. In the case of capacity development, currently four master students are enrolled in Yokohama City University in order to carry-out basic and applied wheat research for future Afghanistan wheat improvement.

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Wheat Inf. Serv. 116: 12-14, 2013. www.shigen.nig.ac.jp/ewis

Research Opinion & Topics

Making Afghanistan wheat secure by 2022

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Afghanistan has been having highly erratic wheat production trend ranging from a high of over five million tonnes in 2009 and 2012 to a low of 2.8 in 2008 and 3.3 in 2011 (MAIL, 2012). However, the Afghan population has been growing steadily and added 10 million during last decade to grow to about 28 million presently. Afghanistan depends on neighbouring countries like Pakistan, Kazakhstan, Iran etc., to meet its wheat needs. Ministry of Agriculture, Irrigation & Livestock (MAIL) has estimated that Afghanistan would need about seven million tonnes wheat by 2022 to achieve self sufficiency. The prospects of achieving two million tonnes jump in wheat production looks gloomy under present scenario where only 45% of wheat acreage is irrigated, which is the major source of wheat production in the country. Rainfed wheat has contributed 10 to 30% of total wheat production in past (Fig. 1), thereby ruling out a reliance on rainfed acreage to achieve self sufficiency in wheat production.

Rainfed wheat contributes substantially to country's wheat harvest during good rainfall years like 2009 and 2012. The rainfed wheat farmers tilling 55% of national wheat acreage needs to be supported with all the available technology and policy support, however, this production domain can not be a reliable source of wheat production for ensuring food security to 35 million Afghans in 2022. During last ten years, rainfed wheat has contributed about 24% of total wheat production in the country (FAO, 2013; MAIL,

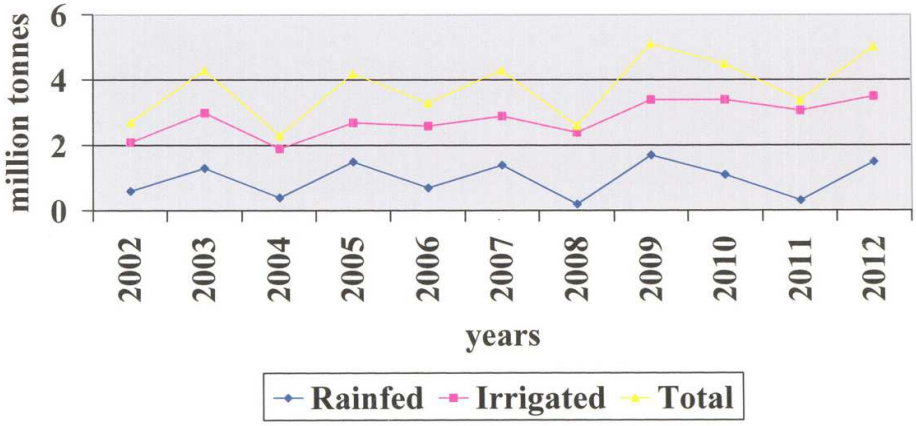

Fig. 1. Rainfed, irrigated and total wheat production in Afghanistan.

2012). One could estimate rainfed wheat to contribute 0.7 million tonnes if the minimum of 10% was expected from rainfed wheat or about 1.68 million tonnes if a more moderate ten year average of 24% was expected. Assuming a more conservative 17% (average of 10 and 24%) contribution from rainfed wheat i.e. 1.19 million tonnes, the irrigated wheat would have to contribute 5.81 million tonnes (Fig. 2) in 2022 to achieve the self sufficiency target. The most viable option to increase wheat productivity in Afghanistan is to bring more farmland under irrigation using the best available technology. Afghanistan brought 28,000 ha under irrigation in 2012 and therefore irrigated wheat acreage could be expected to grow by about 7% every three years. Concerted efforts need to be made to bring more area under irrigation. Proven available technologies like sprinkler irrigation should be promoted to provide supplementary irrigation wherever possible. A quick beginning can be made in districts like Ashkamish, Kalfgan, Khanabad, and Bakwa where water table is quite high. With this projected increase in area, yield per irrigated hectare would still need to increase to 4.1 metric tonnes for Afghanistan to be self-sufficient in wheat in 2022 (Table 1).

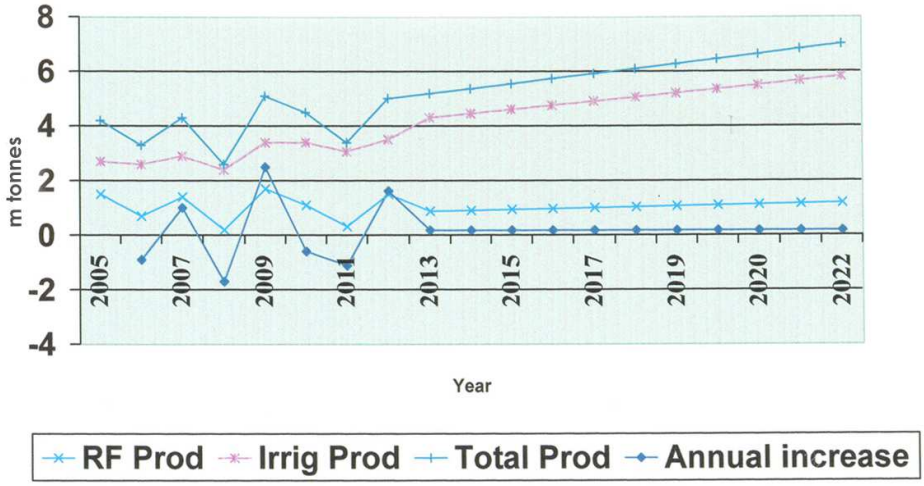

Fig. 2. Rainfed and irrigated wheat production (m tonnes) trend (actual) from 2005 to 2012 and targets for 2022 with 17% share from rainfed.

Table 1. Target yield level by 2022 if irrigated area increases by 7% every three years

Year	No increase in Irrigated Area (000 ha)	7% increase in irrigated area every three years ('000 ha)	Yield (t/ha) needed if there is no increase in irrigated area over 2012 level	Irrigated wheat production needed to achieve total 7 million tonnes if rainfed contributes 17% (000 tones)	Target irrigated wheat yield if 7% increase in irrigated area every 3 year (t/ha)
2016	1167	1249	4.0	4710	3.8
2019	1167	1336	4.5	5260	3.9
2022	1167	1430	5.0	5810	4.1

A multipronged strategy consisting of following components is needed to achieve this target:

1. **Seed System**: Strengthening of seed system to offer farmers more new varieties and discourage the use of less productive, susceptible varieties. The high price of seeds is another barrier to the use of new, more nutrient use efficient varieties. Suggested ways to address this problem include educating farmers about the optimum seed rate of sowing (for example, through the use of the 2012 wheat fact sheet published by CIMMYT and MAIL), and increasing wheat farmers' seed replacement rate from present less than 10% to at least 25%.

2. **Better Crop management**: Advising farmers on how to maximize production and minimize cost of production. It should cover tips on avoiding post-harvest losses; introducing a price support system for wheat; providing affordable credit and crop insurance; and providing other farming inputs in good quality and sufficient quantity at affordable prices.

3. **Research and Extension System**: Strengthening of the national research system (including by hiring dedicated wheat specialists) and of extension services to disseminate research results.

Afghanistan is in dire need of restructuring not only its national agricultural research system but also extension services, input supply chain, marketing infrastructure, credit availability and some kind of post harvest price protection system. A systematic approach to address these issues will definitely enable Afghanistan be wheat sufficient by 2022.

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Wheat Inf. Serv. 116: 15-16, 2013. www.shigen.nig.ac.jp/ewis

Topics on Wheat Genetic Resources

The third term of the National BioResource Project-Wheat, Japan: The first year

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Key words: wheat, genetic resources

The National Bioresource Project-Wheat (NBRP-Wheat) was launched by the Ministry of Education, Culture, Sports, Science and Technology, Japan in 2002 with the aim of maintenance and distribution of seed stocks and DNA clones of wheat. Additionally to its primary roles in collecting, maintaining, and distributing valuable genetic resources, the second-term NBRP-Wheat (2007-2012) featured the collection and characterization of DNA markers.

During the two terms, we have successfully built up a system to maintain the wheat genetic resources under the leadership of Dr. Takashi R. Endo. Our seed collection has around 13,000 accessions. Thanks to Dr. Ogihra's continuous and comprehensive analyses, we have the largest cDNA libraries (1.3 million clones) of wheat in the world. The registered genetic resources are distributed to the users upon request through our database KOMUGI (http://www.shigen.nig.ac.jp/wheat/komugi/top/top.jsp).

The seed stocks of the NBRP-Wheat are being examined for morphological and genomic characters before seed propagation and conservation. NBRP-Wheat is in charge of wheat (genus *Triticum* and genus *Aegilops*) and other related species, rye (genus *Secale*) and small number of the related species in the tribe Triticeae as references. NBRP-Wheat was originally assigned to a core group consisting of the Graduate School of Agriculture, Kyoto University (Laboratory of Plant Genetics and Laboratory of Crop Evolution, whose Collection Code is LPGKU and KU, respectively), Kihara Institute for Biological Research, Yokohama City University (Collection Code: KT), and the Faculty of Agriculture, Tottori University (Collection Code: TACBOW). The TACBOW stocks are now maintained and distributed from Kyoto University. DNA clones are maintained by the Kihara Institute for Biological Research, Yokohama City University. Collectively, we form a network to maintain and distribute the wheat genetic resources that had been stored by individual researches in Japan.

In the second stage of NBRP-Wheat (2007-2012), in addition to maintain and to distribute the genetic stocks, we aimed to collect polymorphic DNA markers that would be useful in genetic studies and wheat breeding. The fruit of the DNA marker project is publically accessible at the KOMUGI database.

At the beginning of the third term (2013-2018), some changes in organization of NBRP-Wheat were made. Namely, we refreshed the member of the steering committee that is formed by leading wheat researchers in Japan to support the activities of the core-facilities (Kyoto University and Yokohama City University). The members of the core-facilities were stepped down from the steering committee to keep the committee's independence. The office of NBRP-Wheat is virtually established in Graduate School of Agriculture, Kyoto University that functions as

management center of the NBRP-Wheat; the tasks are distribution of seed stocks, preparation of documents, arrangements of biannual meetings of the steering committee. And finally, I take the responsibility as the project manager.

The third term of NBRP-Wheat is planned to continue until 2017 to achieve the best collection of wheat genetic stocks in the world. We will establish core-collections of our hexaploid, tetraploid and diploid wheat species. We will modernize the seed storage system in preparation to retirement of Drs. Takashi R. Endo and Taihachi Kawahara who have been enthusiastically maintained the genetic and diversity resources, respectively. It is my sincerest wish to go through this difficult period with the help from world-wide wheat researchers, to be one of the best genetic resource centers of wheat, and to pass the genetic resources to the next generation. Bon voyage!

Wheat Inf. Serv. 116: 17, 2013. www.shigen.nig.ac.jp/ewis

Research Opinion & Topics

The report of National Bioresource Project-Wheat III. Seed resources, 2012

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Maintenance of seed resources: Regenerated seeds of total of 1,559 strains were harvested until early summer in 2012. Four hundred bread wheat were grown in Kihara Institute for Biological Research, Yokohama City University (KIBR) and 1,159 strains consisting of several wild species and landraces were grown in Graduate School of Agriculture, Kyoto University. Total of 1,313 strains were sown in autumn 2012, 314 in KIBR and 999 at Graduate School of Agriculture, Kyoto University.

Distribution of seed resources: Total of 510 strains have been distributed to various researchers and institutions around the world (Table 1).

Table 1. Number of seed stocks distributed in 2013

Code (Institution)*	No. of strains distributed		
	Domestic	Overseas	Total
LPGKU	101	323	424
KU (MOZUME)	61	0	61
KT (KIBR)	24	1	25
TACBOW	0	0	0
Total	186	324	510

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

Wheat Inf. Serv. 116: 18, 2013. www.shigen.nig.ac.jp/ewis

Research Opinion & Topics

National BioResource Project of Japan III: DNA resource of Wheat

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The third phase of the National BioResource Project of Japan "Wheat" has been started in 2012. We, herein, summarize the activity of the first year of the third phase of NBRP-Wheat. DNA resource team of "KOMUGI" has continuously collected, maintained and supplied for cDNA clones of common wheat. In total, 55 cDNA libraries were constructed with the RNAs from stressed tissues as well as various normal tissues. At present, 1,252,563 ESTs (expressed sequence tags) were collected from those cDNA libraries, in which 16,807 full-length cDNA sequences are included. BLAST search of those sequences can be applied from the "KOMUGI" site of National Institute of Genetics, Mishima, Japan. By applying these cDNA sequence data, we constructed the Agilent oligo-DNA microarray harboring 38K gene probes. The wheat oligo DNA microarray is available from the Agilent Co. Ltd.

The plates containing E. coli were shared among four institutions, i.e., Kihara Institute for Biological Research, YCU, Kyoto University, NIG and RIKEN for backup after the 2011 Tohoku earthquake.

In 2012 seasonal activity, 54 clones requested from four institutions inside of Japan were supplied after DNA sequence check.

The work was supported by the National BioResource Project-Wheat from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Wheat Inf. Serv. 116: 19-20, 2013. www.shigen.nig.ac.jp/ewis

Topics on Wheat Genetic Resources

"Core-collection" Project in the National BioResource Project-Wheat, Japan: 2012 Progress report

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Key words: wheat, DNA marker, polymorphism, core-collection

The main aim of National Bioresources Project-Wheat (NBRP-Wheat) is to collect, store and supply wild species, landraces, and experimental strains of wheat and related species. The registered plant materials we currently store are summed up to 13,000 accessions. Of them, 1,500 are genetic stocks mainly composed of aneuploids. The rest 11,000 are the wild species, landraces and historic cultivars. The collections of wild species and landraces derive from several expeditions since Dr. Kihara's first expedition in 1955, and hardly can be collected at the sites today. These accessions have been widely used in evolutionary and diversity studies worldwide, which won high reputation by their sure taxonomic identification and the purity guaranteed by successive self-pollination. Although the data on collection site and pedigree are well documented at the Laboratory of Crop Evolution (the former Plant Germplasm Institute), Graduate School of Agriculture, Kyoto University, the genetic diversity and phenotypic variation are scarcely recorded. In the emerging genomics era, our stocks will be more important than before in search for valuable phenotypic traits. We are surely going to be able to evaluate and utilize the genetic diversity *ex site* conserved in the accessions.

Establishment of a subset of the accessions that represent the total genetic diversity in the collection is desired, which is often referred as core-collection. A number of research papers have been published that deals with establishment of core-collections, large-scale genotyping, and finding marker-trait associations in wheat.

In the third term of NBRP-Wheat, we decided to establish core collections of hexaploid wheat including *Triticum aestivum* and exotic hexaploid wheat, tetraploid wheat (both wild and cultivated, AABB and AAGG species), and diploid wheat (*T. monococcum*, *T. urartu*, and *T. boeoticum*). Here we report the progress made in the fiscal year 2012.

Hexaploid wheat core-collection

We first planned to genotype whole collection of AABBDD species (about 3,500 accessions) by a DArT system. After consideration of seed propagation schedule, we decided not to genotype whole collection at a time, but instead extract DNA samples from around 200-400 plants annually propagated. The first version of core-collection, designated as NBRP-Wheat hexaploid wheat core-collection Ver. 1, was selected based on the collection location, taxonomic classification and importance in Japanese breeding program. The 190 accessions were already genotyped by SSR markers and their basic agronomic characters (20 phenotypes: anthocyanin pigmentation in coleoptile and cotyledon, growth habit, heading date, wax (leaf sheath), wax (spike), wax (column), flowering date, maturation date, number of effective tillers, plant height, spike length, column length, number of spikelet/spike, awnedness, spike color, grain color, glume hair, spike morphology, and 100 kernel weight) year 2011. We asked 12 domestic wheat researchers to test phenotype of their expertise. We obtained genotype data for 186 of

190 accessions based on the DNA microarray technology provided by DArT. The genotype data is analyzed and will be published elsewhere. We had a meeting on the core-collection on December 20th 2012 at Kyoto University to report the progress of core-collection project, and to discuss future plans.

Tetraploid wheat core-collection
We finished isolation of the DNA samples from 2,008 AABB species and 401 AAGG species. The collection site data of these lines are in our hands. We are adjusting the DNA concentration for the genotyping with small number of markers in DArT array format.

Diploid wheat core-collection
We finished DNA preparation for the 284 AA genome species.

Wheat Inf. Serv. 116: 21-24, 2013. www.shigen.nig.ac.jp/ewis

Meeting Reports

The Report of the 7th International *Triticeae* Symposium

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The seventh International Triticeae Symposium (7th ITS) was held at Sichuan Agricultural University in Chengdu, China from 9 to 13 June 2013. The symposium focused on the studies on tribe Triticeae. This tribe includes about 30 genera including the most important cereal crops (wheat, barley and rye). So far, the symposium had been held in Helsinborg (Sweden), Logan (USA), Aleppo (Syria), Cordoba (Spain), Prague (Czech Republic) and Kyoto (Japan).

About 120 researchers and students from 15 countries took part in this symposium. Participants including China transported from the Jinqiang Huaheng Hotel to venue by two motorcoaches and a microbus every day. Such transportation system was like a training camp however, I believed the system encouraged participants to communicate each other. There were 42 oral presentations and near 30 posters. They were grouped in 4 sections, namely Systematics and Phylogeny, Biodiversity and Conservation, Genetics and Genomics, Breeding and Utilization. Here I report some presentations focused on wheat, barley and rye from each sections.

Systematics and Phylogeny

Aegilops tauschii is the D genome donor of hexaploid wheat (*Triticum aestivum*: 2n = 6x = 42, DDAABB genome). "Genome wide survey of SNPs reveals the genetic structure of *Aegilops tauschii*, the D genome progenitor of wheat" by J. Wang, M. Luo, Z. Chen, F. M. You, Y. Wei, Y. Zheng and J. Dvorak reported population structure of 75 *T. aestivum* and 402 *Ae. tauschii* accessions. Genome wide SNP assay was used to genotype the accessions, in which 7,186 mapped SNPs represented 4.02 Gb D genome sequences. They pressed the points that *Ae. tauschii* was consisted of two lineages (Lineage 1 and Lineage 2) and that gene flow between the two lineages were very limited. They revealed that each lineage was consisted of two sub-lineages, that one sub-lineage within Lineage 2 from southwestern and southern Caspian was genetically more similar to D genome of wheat and that *Ae. tauschii* within Lineage 1 was contributed to only 4.4 % of genetic diversity of *T. aestivum*.

Ae. tauschii has two subspecies , ssp. *tauschii* and ssp. *strangulata*. "Intraspecies divergence of *Aegilops tauschii* Coss. As revealed by variation of chloroplast DNA non-coding sequences" by A. J. Dudnikov discussed evolutionary history of *Ae. tauschii* based on sequences of four chloroplast DNA non-cording regions, about 3,000 bp. He argued that *Ae. tauschii* originated in Caucasia and then differentiated into ssp. *tauschii* and ssp. *strangulata* at the beginning of its evolution. He considered that ssp. *tauschii* first start to expand its geographical distribution and rapidly occupied a vast area and that, in contrast to ssp. *tauschii*, ssp. *strangulata* lasted as a small isolated population for a long time span. Several form of ssp. *strangulata*, better adapted to relatively more moist and cool habitats, had originated within the isolated population and has gradually forced out ssp. *tauschii* from some part of its area in the west.

"Biodiversity in the *Hordeeae*: Shearing what is known and identifying what is not known" by M. E. Barkworth, E. Cabi, H. Al-Newani and C. Dyreson introduced web site platform, which uses SYMBIOTA software designed by Ed Gilbert of

Arizona State University. The web site intends to share information accumulated in herbariums around the world (openherbarium.org).

Ae. speltoides is the B and G genome progenitor of tetraploid and hexaploid wheat. "Two genetic lineages in *Aegilops speltoides* – How many refugia in *Aegilops* species?" by T. Kawahara, N. Karashima and S. Takenaka examined genetic diversity of *Waxy* and *Pgk1* genes in 142 *Ae. speltoides* accessions. They revealed that both genes divided in two groups and that combinations of each genetic group were consisted to expectation of random cross. So they concluded that the two different genetic groups do not represent genetic differentiation within species but show gene lineages in each locus. They believed that the two gene lineages would suggest the two refugia, possibly Black Sea coast and Mediterranean coast, during the Last Glacial.

"Novel traits of *Leymus* species that may contribute on wheat improvement" by H. Tsujimoto reported wheat lines with a pair of chromosomes of *Leymus recemosus*, *L. mollis* or *Psathyrostachys huashanica*. His presentation indicated that some alien chromosome addition wheat line showed high phosphorus efficiency and good growth under low phosphorus condition. To apply these traits of the addition lines to wheat breeding, transfer the chromosome arm or segment to the wheat genome is needed. The presentation further showed that a double monosomic addition line carrying semi-homologous chromosomes of *L. recemosus* and *L. mollis* could associate in the meiotic prophase but recombination does not occur between them. The line has good potential to survey the recombination-enhancing factor in wheat.

"Forming of Chinese wheat Species" by N. P. Goncharov discussed about three new polyploid wheat species discovered and/or described since the middle of the last century in China, *T. petropavlovskyi* Udacz. Et Migusch., *T. yunnanense* King and *T. tibetianum* Shao. He believed that *T. petropavlovskyi* is hybrid species carrying the *T. polonicum* gene *P1* for long glumes. He further thought that *T. yunnanense* and *T. tibetianum* should be described as subspecies of *T. spelta* because spelt phenotypes in *T. spelta*, *T. yunnanense* and *T. tibetianum* was controlled by non-allelic genes.

Biodiversity and Conservation

R. von Bothmer, O. Westengen and S. Jeppsson introduced the Svalbard Global Seed Vault in "Is there a need for the Svalbard global seed vault and are our genetic resources safe for the future? Examples in the *Triticeae*". The Global Seed Vault was built as a backup center for international plant genetic resources in 2008. The Vault is located in the arctic area at 78 ˚N, the Norwegian island of Spetsbergen. The Vault has an extra cooling device for keeping the temperature down to -18˚C, standard for gene bank material. In case there are serious breakdown of cooling device, the Vault in permafrost can keep the temperature down to -5˚C.

"English translation of the Russian 'Flora of Cultivated Plants. Wheat' (Dorofeev et al. 1979): project progress report" by H. Knüpffer, A. A. Filatenko, K. Hammer, C. Jeffrey, T. Kawahara and L. A. Morrison reported progress of English translation project of the most recent taxonomic monograph of *Triticum* L. (Dorofeev VF, Filatenko AA, Migushova EF, Udaczin RA, Jakubziner MM. 1979. Flora of cultivated plants. Vol. 1. Wheat. Leningrad, 347 pp.). This important monograph is little known outside of Russia due to the language barrier. The translation project started in 1999 and was funded by CIMMYT. Publication of the translation by Springer (Vienna-New York) is projected for 2014.

"*Aegilops* conservation and breeding potentials" by V. Holubec, A. Hanzalová, V. Dumalasová and P. Bartoš evaluated agronomic important traits of *Aegilops* accessions (including 21 species, 1,082 accessions), which are conserved in the Gene Bank Prague, and 120 new introduced accessions. They investigated resistance for three different leaf rust races and three different stem rust races. They reported that some *Aegilops* species have resistant at least for one leaf rust race at high rates: *Ae. speltoides* (100%), *Ae. lorentii* (*Ae. biuncialis*, 91%) and *Ae. triuncialis* (88%). The same was at least for one stem rust race: *Ae. speltoides* (100%), *Ae. cylindrica* (97%), *Ae. triuncialis* (97%), *Ae. neglecta* (95%) and *Ae. geniculata* (95%).

"Evolution of emmer wheat based on the variations of 5'UTR region of *Ppd-A1*- evidences of gene flow between emmer and timopheevii wheat" by S. Takenaka and T. Kawahara discussed about genetic diversity and evolution of tetraploid wheat based on *Ppd-A1* gene and its around DNA sequences. They showed that a part of domesticated emmer wheat was derived from introgression from wild emmer wheat around Israel. They further showed that there were multiple gene flows between emmer and timopheevii wheat under natural condition.

Genetics and Genomics

Common wheat is an allohexaploid species with the genome size of 17 Gb and 90% repetitive sequences. The largeness and complexity daunted to sequence the wheat genome-based scale. So in 2005, the International Wheat Genome Sequencing Consortium (IWGSC) was established and has proposed to map and sequence the wheat genome based on chromosome-by-chromosome approach. "A 1-Gb leap towards the hexaploid whet genome sequences" by F. Choulet, N. Glover, L. Pingault, J. Daron, S. Theil, N. Guilhot, A. Couloux, V. Barbe, A. Alberti, M. Alaux, P. Leroy, H. Šimková, J. Doležel, A. Bellec, H. Bergès, P. Sourdille, E. Paux, H. Quesneville, P. Wincher and C. Feuillet reported progress of the sequencing project of chromosome 3B (1 Gb). They sequenced 8,452 BAC clones of the MTP using NGS technology and obtained 4,999 scaffolds (N50 = 463 kb). They have produced a pseudomolecule covering 775 Mb anchored to genetic and phenotypic maps with more than 4,000 markers.

"Characterizing the structural and functional variations of the bread wheat chromosome 3B gene space" by P. Lise, C. Frederic, T. Sébastien, G. Natasha, S. Pierre, L. Philippe, G. Nicolas, B. Francois, A. Adriana, C. Arnaud, B. Valerie, IWGSC, Van del P. Klaas, W. Patrick, F. Catherine and P. Etienne reported fine transcription map based on a pseudomolecule described above and information of RNA-seq. They showed that 82% of the 6,812 predicted genes were expressed, with more than half displaying alternative splicing. 2,724 genes were expressed in the 15 conditions and 6% of the genes showed a condition-specific profile. They also reported that 2,393 new transcriptional active regions were identified in non-annotated regions.

"Using physical map and survey sequences to unravel the structure and composition of wheat chromosome arm 7DL" by L. Wang, X. Nie, H. Šimková, J. Safar, J. Doležel, E. David, M. Luo and W. Song reported progress of the sequencing project of chromosome 7DL carried out in the framework of IWGSC. A total of 50,304 clones were fingerprinted. An assembly resulted in 1,614 contigs (N50 = 296.4 kb) and 4,472 MTP clones were generated from the assembly. They showed that 846 contigs of wheat 7DL chromosome were anchored based on *Ae. tauschii* physical map. Furthermore, they reported that they survey sequenced 7DL-DNA using NGS technology and gained a total of 14.54 Gb (70x) to date. After de novo assembly, they obtained 160,021 contigs with the total base of 223 Mb (covering 65% of 7DL chromosome).

"Development of PCR based PLUG markers specific to individual rye chromosome arms" by J. Li, T. R. Endo and S. Nasuda reported PCR based markers for rye chromosome. The PLUG markers were selected from wheat PLUG markers whose chromosomal regions had been determined. Based on the homology between wheat and rye chromosomes, they showed complex structural rearrangements between them.

A plant cuticle, which is composed of cutin and wax, covers the surface of land plants and prevents uncontrolled water loss. "Cuticle mutants and gene isolation in barley (*Hordeum vulgare*)" by G. Chen, C. Li and T. Komatsuda investigated a cutin defective mutant of barley (*eibi1*). The mutant had thin cuticle and a low capacity to retain leaf water. They showed that *Eibi1* encoded an *HvABCG31* full transporter by genetic mapping and thought that the mutant was inhibited transportation of cutin. Furthermore they investigated *eceriferum* (*cer*) mutants, which had reduced or absent epicuticular wax crystal, and assigned the mutants to 79 loci. One of *cer* mutants, *cer-zv*, showed a significant reduction in cutin monomers but not wax therefore *cer-zv* mutant is the second cutin defective mutant. They showed *cer-zv* was located in the centromeric region of chromosome 4H.

"Major variation in spike form in the Triticeae species" by T. Komatsuda discussed morphology of spike of barley (two- or six-row). The differences are controlled one gene *Vrs1*, which encodes homeodomain-leucine zipper I class transcription factor and is a paralog of *HvHox2*. He showed the differences of expression patterns and functions between *VRS1* and *HvHox2*. The transcriptional activation activity of *VRS1* and *HvHox2* was conserved but expressed in different places. He thought that function of *Vrs1* is to inhibit gynoecial development because the transcription level of *Vrs1* was more than ten-fold greater than that of *HvHox2* during the pistil development stage. Furthermore he reported that *Vrs1* expression was up-regulated by *Vrs4* (*vrs4* is another six-row mutant).

Breeding and Utilization

Wheat is an allopolyploid which has two or more sets of related chromosomes. Despite their genome complexity, wheat behaves as diploid during meiosis because *Ph1* locus, located on chromosome 5B, prevents pairing between homoeologous chromosomes. "Uses of the *ph1* mutations as a genetic tool for breeding" by M. D. Rey, M.C Calderón and P. Prieto reported development of new wheat alien chromosome substitution line carrying chromosome segments

from *H. chilense*. They first showed that inter specific recombination between wheat and *H. chilense* promoted efficiently in the background of the *ph1* mutants.

"The responses of germinating barley seeds to salt stress" by W. Xue, J. Yan, X. Zhao, R. Wang, F. Tzion, A. Korol and J. Cheng proposed new index for evaluation of salt tolerance of crop. Salt tolerance is one of the most important traits of crop breeding. However, researches on the mechanisms of salt tolerance of seedlings are relatively poorer than those in growing plants. They invented index to evaluate seed germination responses to salt stress and applied the index to both Chinese barley cultivars and Israeli wild barley (*H. spontaneum*). The Chinese barley cultivars exhibited diverse salt tolerances with the values ranging from 29.1 to 310 mM (NaCl solution) and the Israeli wild barley exhibited high tolerances from 340 to 450 mM.

In recent years, the studies on barley and wheat have progressed rapidly. In 2010, whole genome sequencing of *Brachypodium distachyon*, a model plant for temperate grasses, finished and we can use the information for research on Triticeae (IBI, 2010). In Triticeae, most parts of barley (*H. vulgare*) genome have been sequenced (IBSC, 2012). In 2013, moreover, physical maps of *Ae. tauschii* and *T. urartu* have been reported (Luo et al. 2013, Ling et al. 2013). Because of the very large and complex genome structure, whole genome sequencing of wheat had been considered difficult. However, now the genome project of wheat has progressed under the initiative of IWGSC and the results were reported in this symposium. In this symposium, many researches on interspecific variation and phylogenetic evolution of perennial plant (*Elymus*, *Leymus* etc.) were presented. Further studies are needed because many questions are left unsolved regarding to the evolution and distribution of Triticeae.

The symposium was nicely organized under the joint auspices of Local Organizing Committee chaired by Prof. Yen Chi, International Organizing Committee and National Natural Sciences Foundation of China. The next International Triticeae Symposium will be held at IPK (Gatersleben, Germany).

Finally, My travel to China was supported by a grant from LOC of the 6th ITS. I would like to thank the supports.

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Wheat Inf. Serv. 116: 25-26, 2013. www.shigen.nig.ac.jp/ewis

Others

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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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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) 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.

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