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Research Information

Lalmi 04- A new rainfed wheat variety for Afghanistan

M. Qasem Obaidi[1], M. Hashim Azmatyar[1], Elias Mohmand[2], Assadullah Habibi[3], Abdul Qayum[2], Rajiv Sharma[2]

[1]Agricultural Research Institute of Afghanistan, Kabul, Afghanistan
[2]International Maize and Wheat Improvement Centre (CIMMYT), Kabul, Afghanistan
[3]Food and Agriculture Organization, Kabul, Afghanistan
*Corresponding author: Rajiv Sharma (E-mail: rk.sharma@cgiar.org)

Abstract

A new rainfed wheat variety Lalmi 04 has been released in Afghanistan from out of the genotypes introduced through 14th Semi Arid Wheat Yield Trial of CIMMYT in 2006. The variety was tested in a total of eight yield evaluation trials over a period of four years at drought prone locations across country in North, East as well as West *viz.*, Mazar, Takhar, Jalalabad, Kunduz and Herat. Lalmi 04 gave more than 10% higher yield than the check variety Lalmi 02. The released variety showed an yield potential of 6836 Kg/ha and an average yield of 3826 Kg/ha as compared to an average of 3463 Kg/ha by Lalmi 02. The candidate variety Lalmi 04 had an average 1000 grain weight of 44 grams and matured in 179 days. The variety has an erect flag leaf and grew to a height of 88 cm. The variety was resistant to all the rusts in Afghanistan under natural conditions.

Keywords: Afghanistan, wheat, rainfed, variety, productivity

Introduction

Wheat is the most important food crop of Afghanistan occupying about 80% of total cereal area in the country (APR, 2012). The country does not have a consistent wheat production record as about 55% of the total wheat acreage depends on rains which have been erratic and insufficient during many years in recent past. Afghanistan harvested its highest wheat harvest of 5.1 million tonnes in 2009 and a slightly less of about 5.0 million tonnes in 2012 owing mainly to good rains. Irrigated wheat domain is a more assured supplier of wheat in Afghan agriculture and contributed 70 to 90% of total wheat production during last many years (APR, 2012). Figure 1 shows how country's wheat harvest fluctuates when rainfed wheat falters. However, rainfed wheat as such faces a number of constraints that render its cultivation prone to risk. Lack of sufficient number of improved high yielding disease resistant varieties is one of the reasons. The poor yield levels and opportunistic nature of rainfed wheat shies away rainfed wheat farmers from investing in its cultivation. The country currently has about 16 irrigated wheat varieties in seed chain compared to only three for rainfed wheat. Interestingly these three varieties, catering to 55% of acreage, account only for 33% of total certified seed production plan. Another alarming situation has been the emergence of new yellow rust races to which important rainfed wheat variety viz., Ghori 96 has become susceptible (Zamarai *et al*., 2013) further denting the availability of new certified seed to rainfed wheat farmers. CIMMYT in association with national agricultural research system represented by Agricultural Research Institute of Afghanistan (ARIA) routinely introduces large number of new high yielding and disease resistant rainfed wheat genotypes into the country and jointly test their suitability for Afghanistan in multilocation yield evaluation trials. Based on the results of these trials and on ARIA's proposal, the Ministry of Agriculture, Irrigation & Livestock (MAIL) of the Government of Islamic Republic of Afghanistan (GIRoA) has recently released a new rainfed wheat variety for commercial cultivation for the farmers of Afghanistan. This note reports the performance and adaptability of this CIMMYT genotype to

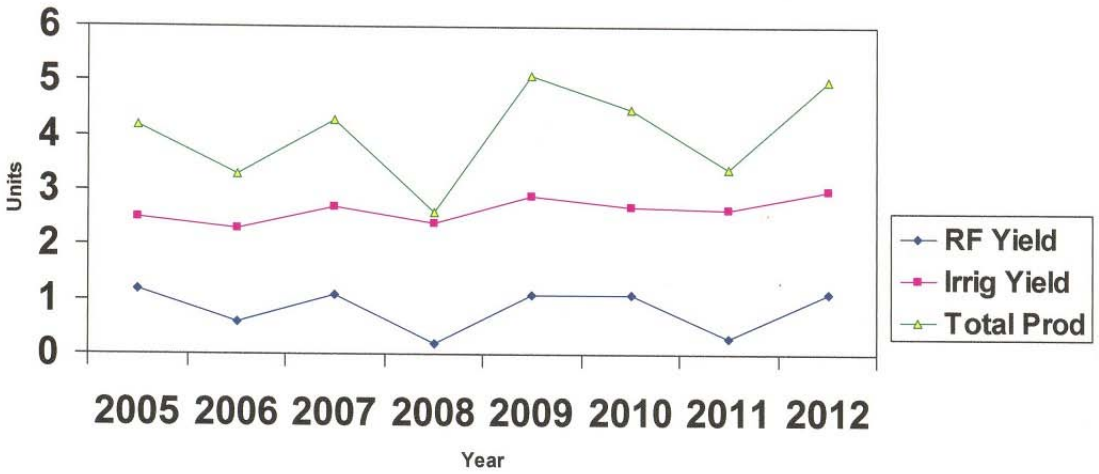

Fig. 1. Rainfed and irrigated wheat yields (tonnes/ ha) and total wheat production (million tonnes) trends in Afghanistan from 2005 to 2012.

Table 1. Average performance of Lalmi 04 during four years of testing as compared to check variety Lalmi 02

Genotype	Yield (Kg/ha)					Superiority (%)
	14th SAWYT (2006-07)	PYT-SA (2007-2008)	NUT-RF (2010-11)	NUT-RF (2011-12)	Average	
14th SAWYT # 324	3325 (3L)	4591	3853 (2L)	4170(2L)	3826	10.4
Lalmi-02/ Best Check	2900	4796	3508	3598	3463	

Afghan conditions and how the variety compared with other existing variety.

Materials and Methods

The variety Lalmi 04 was introduced into Afghanistan through 14th Semi Arid Wheat Yield Trial (SAWYT) in 2006. The variety was tested for four years (Table 1) at several locations in the country. Lalmi 04 has proven rainfed varieties like Silver Star (from Australia) and Pastor (from CIMMYT, Mexico) in its pedigree [SLVS (Sliver Star)*2/ PASTOR] and was tested in 14th SAWYT at Jalalabad, Mazar and Takhar during the crop season 2006-07. The SAWYT trial comprising of 50 genotypes was laid in alpha lattice design with two replications. The following year Lalmi 04 was tested in Preliminary yield trial- semi arid (PYT-SA) at Kunduz in completely randomized block design (CRBD) design with three replications. However, during next two years, the variety could not be tested and it entered wheat yield evaluation trials again in 2010-11 and was tested in National Uniformity (NUT) trial at two locations of Herat and Takhar. The genotype was tested once again in NUT during 2011-12 at Takhar and Mazar. Both the NUT trials were conducted in CRBD with three replications. All the trials were sown in a timely fashion using the standard seeding rate (105 kg/ha). Individual experimental plots of 6.0 m^2 were seeded as six rows with 0.20-m row spacing. In addition to yield potential, the variety was also evaluated for other important traits like height, days to maturity and disease reaction. The variety was also screened for rust reaction at Njoro Kenya as part of National Rust Screening Nursery (NRSN) during 2010-11. In all the yield evaluation trials, the locally recommended agronomic practices were adopted.

Results and Discussion

Good wheat harvest in Afghanistan depends on good rainfed wheat crop. Though irrigated wheat is more or less consistent in yield, however, is unable to produce sufficient for the country's requirements. Only when rainfed wheat also produces a good crop, the country harvests wheat quantities closer to its self sufficiency needs. The variety Lalmi 04 was introduced into Afghanistan through 14th Semi Arid Wheat Yield Trial (SAWYT) in 2006. The variety was found superior to existing check Lalmi 02 by about 10% and was thus released in the variety release committee meeting in January, 2013. During the first year of testing, Lalmi 04 yielded an across

Table 2. Description of Lalmi 04 based on evaluation in Afghanistan

Parameter	Variety
Parentage / Source	SLVS*2/ PASTOR
Growth Class	Spring
Growth Habit	Semi prostrate
Plant Height (cm)	88
Lemma (awn/hood)	Awned
Grain Color	White
Days to heading	140
Days to Maturity	179
Auricle color	White
Flag Leave Attitude	Erect
Flag Leave Width	Medium
1000 Kernel Wt(g)	44
Yield Potential(Kg/ha)	6836
Average Yield (Kg/ha)	3826

location mean yield of 3325 Kg/ ha against Lalmi 02 which yielded 2900 Kg/ ha in SAWYT. The following year in PYT-SA at Kunduz, Lalmi 04 yielded 4591 Kg/ ha, lower than 4796 Kg/ ha of Lalmi 02. However, during 2010-11 and 2011-12, this variety yielded higher than the check and achieved an overall superiority of over 10% against the check Lalmi 02. Its across location mean yield stood at 3853 Kg/ ha in 2010-11 and 4170 Kg/ ha in 2011-12 against 3508 Kg/ha and 3598 Kg/ha of Lalmi 02, respectively. The variety recorded no rust in Afghanistan during four years of testing whereas under artificial epiphytotic conditions at Njoro, Kenya, Lalmi 04 was observed to have 10M stem rust and 10-15 M yellow rust. Lalmi 04 has an average plant height of 88 cm and mature in 179 days. It has an erect flag leaf and 1000 grains weigh 44 grams. Lalmi 04 showed an yield potential of 6836 Kg/ ha and an average yield potential of 3826 Kg/ ha compared to 3463 Kg/ ha of Lalmi 02 showing over 10% yield superiority over four years of yield evaluation trials. The other distinguishing features of Lalmi 04 are presented in Table 2. The release of this variety for commercial cultivation addresses the critical gap of lack of suitable rainfed varieties in the country. This issue has assumed alarming proportion with Ghori 96, a highly popular rainfed variety, falling susceptible to yellow rust recently (Zamarai et al., 2013). Though rainfed wheat yields remain opportunistic in nature, release of this variety offers farmers a high yielding and disease resistant option.

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Research Information

Multiple disease resistance in wheat: Need of Today

Pooja Sharma[1], R. B. Sharma[1†, 2], Amit K. Singh[3], Rakesh Singh[3], Sundeep Kumar[3]

[1]Shri Vankateshwara University Vankateshwara Nagar, Gajraula, J.P. Nagar – 244236, India
[1†]Visiting Professor, Shri Vankateshwara University Vankateshwara Nagar, Gajraula, J.P. Nagar – 244236, India
[2]Department of Botany, Saraswati (P.G.) College, Hathras, U.P., India
[3]Food and Agriculture Organization, Kabul, Afghanistan National Bureau of Plant Genetic Resources, New Delhi-110012, India
*Corresponding author: Pooja Sharma (pooja_genome@rediffmail.com)

Wheat as staple food of about 35% world population is facing difficulty due to growing problem of multiple biotic stresses under changing climate scenario. Leaf rust, stem rust and spot blotch are the major biotic stresses of wheat (*Triticum aestivum* L.). According to Park (2007), leaf rust alone can cause up to 60% yield losses while stem rust can cause 100% yield losses in severe conditions. The losses due to rusts can be large and can vary from year to year and region to region (Sawhney, 1995). The average yield losses due to leaf blight (Spot blotch) is about 20% in India (Joshi *et al.*, 2004) however, it may be up to 80% under heavy infection (Joshi *et al.*, 2007). Therefore, development of resistant varieties for single disease is not enough to save plant product and to feed growing population of the world. These all diseases together toll heavy yield losses and put us in the situation to redesign our strategies to fulfill our requirements. The majority of currently grown wheat varieties are susceptible to these diseases, presumably because of high pathogenic variability occurring in these fungi and narrow genetic base for resistance in currently available wheat varieties. Characterization of our existing germplasm for multiple disease resistance varieties may be a vital option in this regard. The grown varieties on the market do carry some known disease resistance genes against rusts and low levels of quantitative resistance against spot blotch diseases. However, most of the resistance genes against rusts are already broken down. Information regarding spot blotch resistance is not well documented. In the sustainable agriculture, which is economical both for the farmer and nature, multiple disease resistance is an essential tool against pathogens attack beside cultural practices, like crop rotation (McIntosh, 1998). With the biotrophic fungi like rusts, the only solution is the durable disease resistance.

During the last 50 years, significant improvement in wheat production and productivity was achieved through exploitation of major genes for traits like dwarfness, photoperiod insensitivity and resistance to biotic stresses (Reynolds and Borlaug, 2006). However, a quantitative jump in wheat production and productivity is still needed to feed the fast expanding human population despite of drastic changes in world climatic conditions. The current major challenges in the successful wheat production are increasing heat stress, dwindling water supplies for irrigation, a growing threat of new virulence of diseases such as wheat rusts (yellow, brown, black and stem), spot blotch, continuous adoption of rice-wheat systems on around 11 million hectares, changes in urbanization patterns, and demand for better quality wheat (Joshi *et al.*, 2007).

Leaf rust caused by *Puccinia triticina* (=*P. recondita* Roberage ex Desmaz f. sp. *tritici* Eriks and E. Henn.), stripe rust caused by *P. striiformis* Westend f. sp. *Tritici***,** stem rust caused by *Puccinia graminis* f. sp. *Tritici* and spot blotch caused by *Bipolaris sorokiniana* are considered as most significant diseases in almost all part of the world where wheat is grown (Ginkel and Rajaram, 1998; Singh *et al.*, 1995; Joshi *et al.*, 2007). These are most common disease which causes almost 50% yield loss. Although, reported as early as 1940 by Mehta, it has gained importance after Green Revolution (Singh and Rajaram, 1992). A number of rust resistance genes are known to

provide complete protection (McIntosh *et al.*, 1995) but due to higher rate of breakdown of resistant genes and their narrow genetic base enhance the wheat cultivars susceptibility to rust disease. An epidemic of stem rust on wheat caused by race TTKSK (e.g. isolate Ug99) is currently spreading across Africa, Asia and the Middle East and is causing major concern due to the large numbers of people dependent on wheat for sustenance. The strain was named after the country where it was identified (Uganda) and the year of its discovery (1999) (Singh *et al.*, 2011). *P. graminis* is a member of the Phylum Basidiomycota within the Kingdom Fungi. The characteristic rust color on stems and leaves is typical of a general stem rust as well as any variation of this type of fungus. Different from most fungi, the rust variations have five spore stages and alternate between two hosts. Wheat is the primary host and barberry is the alternate host.

New biotic stresses like foliar blight (spot blotch) has also emerged a big constraint for the successful production of wheat in South East Asia (Wiese, 1987; Mathur and Coufer, 1993). Work on the spot blotch was initiated long back (Mishra, 1973) to find the answer of this emerging disease in South-Asia. Variation in the pathogen population and aggressiveness which increase over time are the major concerns in case of spot blotch (Chand *et al.*, 2003). Numerous, breeding programs have been under taken to understand the nature of resistance and incorporation of resistance in to commercial cultivars (Adalkha *et al.*, 1984). However, resistance to spot blotch in the commonly grown wheat cultivars of South East Asia is generally unsatisfactory (Joshi *et al.*, 2004). This disease is now expending towards non-traditional cooler regions like North West Plain Zone (NWPZ) of India, which is considered as major contributor of wheat in South Asia. The available literature covering various aspects falling under the scope of the present study is being detailed as under:

Host range of pathogen

Wheat, barley, triticale, and a few related species are the primary hosts for leaf rust (*Puccinia recondita*), stem rust (*P. graminis* f.sp. *tritici*) and spot blotch (*Bipolaris sorokiniana*). The primary alternate host in nature has been *Berberis vulgaris* L., a species native to Europe, although other species have been susceptible in greenhouse tests in case of rusts while, spot blotch appears on host species and survives in soil on crop residues during non-crop season. Sprague (1950) reported that *B. sorokiniana* has a large host range and almost all the plants belonging to family *Poaceae* come under its host range. Apart from wheat, it infects oat, barley, rye, *Phylaris*, *Agropyron*, *Pennisetumm*, *Lollium*, *Poae*, *Secale*, *Setaria* etc. (Bakonyi *et al.*, 1998).

Survival

Weather conditions and age of the plants during late October till December are quite favorable for infection by rust fungi. However, in north India initial outbreaks of rust are delayed by 2-3 months, in case of leaf rust. This suggests that there is no local source of primary inoculum of any of the rust of wheat. In the plains of north India due to prevailing high temperatures after harvest of wheat crop, during the summer months, the urediospores and teliospores of the fungus are killed. Leaf rust may survives between crops as mycelium or as uredinia on infected volunteer and/or on early sown and late maturing wheat crops or native grasses until a fresh crop of wheat is available (Chester, 1946; Eversmeyer and Kramer, 2000). Like other *Puccinia* species, *P. graminis* is an obligate biotroph and has a complex life cycle featuring alternation of generations. The fungus is heteroecious, requiring two hosts to complete its life cycle - the cereal host and the alternate host (Schumann *et al.*, 2011). There are many species in *Berberis* and *Mahonia* that are susceptible to stem rust, but the common barberry is considered to be the most important alternate host (Singh *et al.*, 2008) *P. graminis* is macrocyclic (exhibits all five of the spore types that are known for rust fungi (Schumann *et al.*, 2011). The pathogen can survive almost any condition the host leaf can survive (Mehta, 1940). In case of spot blotch, a humid cloudy weather condition favours the survival and spread of disease (Chaurasia *et al.*, 1999).

Occurrence

There are several areas worldwide in which each of the rusts can cause severe losses (Saari and Prescott, 1985). *Puccinia triticina* can survive the same environmental conditions that the wheat leaf survives, provided infection but no sporulation has occurred. The fungus can infect with dew periods of three hours or less at temperatures of about 20°C, however, more infections occur with longer dew periods. At cooler temperatures, longer dew periods are required, for example, at 10°C a 12 hours dew period is necessary. Few if any infections occur where dew period temperatures are above 32°C (Stubbs *et al.*, 1986) or below 2°C. Once the leaf has become infected, temperature dictates the incubation period (Hogg *et al.*, 1966; Eversmeyer *et al.*, 1980). The epidemiology of *P. graminis* is similar to *P. triticina*.

Spot blotch is a disease of importance mainly in warm, humid wheat growing Mega environment (ME-5) where the mean temperature of the coolest month is higher than 17.5°C (Dubin *et al.*, 1998). It causes serious yield losses to wheat crop in

India (Joshi *et al.*, 2002), South East Asia (Saari, 1998), North and Latin America, Africa (Duczek and Jones-Flors, 1993), China (Chang and Wn, 1998) and Brazil (Mehta, 1993). More recently, spot blotch has also expended into the cooler, non-traditional irrigated rice-wheat production areas (ME-1) (Dubin and Ginkel, 1991; Duveiller and Gilchrist, 1994; Ginkel and Rajaram, 1998).

Inoculum Source and Infection

The main methods of inoculation include: dusting or brushing with dry spores**,** with or without a carrier such as talc or spores of the club moss *Lycopodium*; spraying with water or isoparaffinic oil-based suspensions**;** or plant tissue injection using water-based suspensions. Inoculation by uredinia involves regional transport of urediniospores by wind (Hirst and Hurst, 1967; Watson and de Sousa, 1983), may introduce a new virulent race in an area, but probably seldom results in a severe epidemic that season except on the most susceptible cultivars. Aeciospores from the alternate host (sexual) rarely result in an epidemic. The urediospores of *Puccinia triticina* are brown and spherical, 16-28 microns in diameter and with a minutely echinulate wall furnished with 7-10 germ pores. Infection by germ tubes from urediospores occurs through stomata on either side of the leaf. The germination process requires moisture, and works best at 100% humidity. Optimum temperature for germination is between 15°C -20°C. Before sporulation, wheat plants appear completely asymtomatic. This is because rust pathogens are biotrophic and require living plant cell to survive.

In case of spot blotch, isolates having dark green to green colour were highly sporulating, while those with grey to white colour were poor in sporulation (Chand *et al.*, 2003). Duczek *et al.* (1996) reported that sporulation of *Bipolaris sorokiniana* varied from year to year. They observed production of conidia of *Bipolaris sorokiniana* on the crowns of field grown annual crops; sporulation was highest in crown region of wheat and other grasses.

Symptoms

Rust fungi all produce similar disease symptoms on the host plants and have similar requirements for infection. The diseases get their name from their appearance on the plant. Infection can occur on any above-ground plant part, leading to the production of pustules that contain thousands of dry yellow-orange to reddish-brown or black spores. These pustules give the appearance of "rust" on the plant.

Stem rust occurs primarily on stems but can also be found on leaves, sheaths, glumes, awns and even seed. Symptoms begin as oval to elongate lesions that are generally reddish-brown in color. In the late stages of the disease, erumpent pustules produce numerous black sooty spores. Severe infestations with many stem lesions may weaken plant stems and result in lodging.

Leaf rust is generally found on leaves but may also infect glumes and awns. Symptoms begin as small, circular to oval yellow spots on infected tissue of the upper leaf surface. As the disease progresses, the spots develop into orange colored pustules which may be surrounded by a yellow halo. The pustules produce a large number of spores that are easily dislodged from the pustule resulting in an "orange dust" on the leaf surface or on clothes, hands and equipment. As the disease progresses, black spores may be produced resulting in a mixture of orange and black lesions on the same leaf. Tiny orange lesions may be present on seed heads, but these lesions do not develop into erumpent pustules. This difference helps to distinguish leaf rust from stem rust.

The symptoms of spot blotch appear as small, light brown lesions which are scattered throughout the leaves and increase in size with stage advancement. Later, these lesions coalesce and change to large spots after a week of infection. These spot are of different size and shape (oval to oblong and measuring 0.5 to 10 mm long and 3 to 5 mm wide). Symptoms of spot blotch are also commonly noticed on leaf, sheath, nodes and glumes (Chand *et al.*, 2002).

Yield Loss

Stakman *et al.* (1962) note that leaf and stripe rust generally do not cause the same level of yield damage as stem rust. However, both typically can become as epidemic as stem rust, and each may cause greater annual damage then stem rust in certain areas. Traditionally, stripe rust is likely to be most destructive in cool, moist seasons; stem and leaf rusts are likely to be most destructive in warm, moist seasons. However, this appears to be changing. In recent years new, higher temperature tolerant, aggressive strains of stripe rust are moving into non-traditional, warmer areas (Hovmøller and Henriksen, 2008; Milus *et al.*, 2009). Hanson *et al.* (1982) provided a summary of the impacts of rust diseases in developing countries and identified the hot spots for each of the rusts. Table 1 indicates that stem and stripe rusts are more destructive in an epidemic, but that leaf rust is more significant endemically.

Yield losses caused by the spot blotch disease are conciderable in South Asia (Dubin and Ginkel, 1991). Saari (1998) reviewed the losses reported for the wheat leaf blight diseases and concluded that average loss due to leaf blight in South Asia was 19.6% (Table 1).

Table 1 Summary of losses caused by Leaf & stem rusts and spot blotch diseases in developing countries.

Rust	Yield loss (%) in susceptible varieties		Endemic areas as proportion of total wheat areas (%)	Hot spots – areas where disease is most severe
	Average in endemic area	In epidemic		
Stem rust	40%	Up to 100%	50%	Highlands of Kenya and Ethiopia; Parana State, Brazil; South India
Leaf rust	15%-20%	Up to 50%	90%	Mexico, India, Pakistan, Bangladesh, China
Spot blotch	19.6%	Up to 80%	-	South-East Asia including India, Nepal, Bangladesh

Genetics of resistance

(a) Rust resistance

A complete study of wheat rust genetics demands the genetics of both host and pathogen. Although genetics of the rust pathogens is difficult to study, the genetics of the host (wheat) can be studied from the interaction between host and pathogen which produce incompatible (resistance) and compatible (susceptible) reactions. Majority of already designated stem rust resistance genes are of dominant nature and under monogenic control (McIntosh *et al.*, 1995). In Wheat (*Triticum* spp.) and leaf rust (*Puccinia recondite* f. sp. *tritici***)** interaction, additive type of gene action have been reported (Singh *et al.*, 1998). Studies with combining ability analysis expressed both additive and non additive gene effects for resistance to leaf rust in wheat (Chawla *et al.***,** 1990). Singh *et al.* (2004, 2005) also reported two to five more genes for leaf rust and stripe rust resistance from many cultivars in addition to *Lr34* and *Yr18* that are contributing towards their durable resistance.

(b) Spot blotch resistance

Efforts have been made to reveal the inheritance of resistance to spot blotch. According to Velazquez Cruz (1994), inheritance of spot blotch seems to be polygenic with additive effect. Kumar *et al.* (2007) reported the involvement of two to three genes in the resistance mechanism of spot blotch. This is in accordance with the previous reports based on Indian wheat genotypes where one or two genes control was reported (Srivastava *et al.*, 1971; Srivastava, 1982; Adlakha *et al.*, 1984). However, these studies did not go beyond F2 generation and utilized limited population size. Further, that time spot blotch was not an important disease of Indian sub-continent and effective pathotypes were also not available for characterization. Later, Joshi *et al.* (2004) reported the involvement of three genes having additive effect in resistance sources *viz.*, Mon/Ald, Acc. 8206 and Suzhoe-8 based on advanced segregating generations and big size populations.

The heritability estimates for these crosses were of moderate type ranging from 0.65 to 0.8. Sharma *et al.* (2007) and Joshi *et al.* (2004) also reported moderate range of heritability with respect to spot blotch resistance. According to Joshi *et al.* (2004) by creating effective afrificial epiphytotic conditions and proper disease severity recording taking care of the growth stages, environmental effect could be minimized thus, higher estimates of heritability can be obtained.

The evaluation of large number of genotypes (*aestivum* and *durum*) and triticale over the year has clearly established that majority of the durum (AB genome) genotypes are highly susceptible to leaf blight pathogen. On the other hand, the *aestivum* (ABD genomes) genotypes fall under the category of susceptible to moderately resistant. Most of the triticales (ABR genome) seem to be moderately resistant to resistant. These data indicated that in *aestivum* wheat, resistance is probably located on D genome and in case of triticale on the R genome (Chand *et al.*, 2002).

Breeding progress for multiple disease resistance

It is assumed that resistant genes effective against prevailing races of a particular region, the chances of yield losses is lesser than those varieties which are not carrying these resistance genes. Chances of out breakage of varieties are more if only one or two resistance genes are present. Enhanced resistance can be achieved by adding some other resistance genes in addition to the existing one. Incorporation of 3-4 prominent resistant genes conferring resistance against leaf and stem rusts and spot blotch diseases chances of out breakage

of disease is low and the duration of efficient resistance can be increased and multiple disease resistance can be achieved (Singh *et al.*, 2000)

Rust pathogens can mutate to overcome the existing resistant genes. Therefore, breeding for rust resistance needs to focus on maintaining current levels of resistance and on developing new and improved sources of resistance. The maintenance of rust resistance involves a continuing search for, and development of, new forms or combinations of resistance, to ensure that the varieties in farmers' fields have effective genetic resistance against the current strains of the pathogens. To ensure that rust does not cause economic losses, breeders need to have an understanding of cultivar susceptibility, the rust pathogens and a sense of the resistance genes available for use.

To achieve this, three or four lines carrying different minor genes were crossed (3-way and 4-way crosses), and plants in large segregating populations were selected under artificially created rust epidemics. Races of pathogens that have virulence for race-specific resistance genes present in the parents were used to create the epidemics (Singh *et al.***,** 2000). The experience of breeders to achieve partial resistance in breeding populations (Dubin and Ginkel, 1991; Duveiller and Gilchirst, 1994; Dubin and Rajaram, 1996) suggested polygenic type of resistance. Breeding for durable resistance based on minor additive genes has been challenging and often slow, for several reasons:

1) A sufficient number of minor genes may not be present in a single source genotype,
2) A source genotype may be poorly adapted,
3) There may be confounding effects from the segregation of both major and minor genes in the population,
4) Crossing and selection schemes and population sizes are more suitable for selecting major genes,
5) Reliable molecular markers for several minor genes are unavailable, and
6) The cost associated with identifying and utilizing multiple markers, is high.

However, such germplasm carrying combinations of minor genes should be very useful in transferring these genes to adapted local cultivars.

Availability for tightly linked DNA markers in the future can be useful in maintaining and diversifying the combinations of additive slow rusting resistance genes in the wheat germplasm and cultivars (Sareen *et al.*, 2012). The actual use of multiple markers in breeding strategy at CIMMYT is likely be limited to the characterization and selection of parents to be used in specific crosses as the field screening is very reliable and cost-effective. However, if such genes need to be incorporated in adapted cultivar that contains an effective race-specific resistance gene, then markers are the only option and will be used despite the cost.

Molecular tools in achieving multiple disease resistance

Molecular tools have opened up new dimensions in the area of crop improvement. In condition of the availability of linked molecular markers, an effective selection strategy can be made for the development of multiple disease resistance varieties. Once, markers are established for different resistant genes conferring resistance against multiple diseases, these markers can be used in selecting multiple disease resistant varieties through marker assisted selection (MAS). There are number of varieties which are not currently being grown due to their susceptibility however, having good in quality and production, can be improved further, through intensive crossing program which following marker assisted selection. The association of leaf tip necrosis with Lr34/Yr18 genes was established by Singh *et al.* (2000). Later, Joshi *et al.* (2004) established the linkage of leaf tip necrosis with spot blotch resistance. In addition, stay green trait was also showed positive linkage with spot blotch resistance (Joshi *et al.*, 2007). These morphological markers are successfully being used in markers assisted selection of spot blotch resistance germplasm lines.

Number of molecular markers linked to leaf and stem rusts resistance genes/QTLs have been identified in wheat (Singh *et al.*, 2011). As far as stem rust is concerned, some of them arose in bread wheat (e.g. *Sr5* and *Sr6*), while others have been bred in from other wheat species (e.g. *Sr21* from *T. monococcum*) or from other members of the tribe *Triticeae* (e.g. *Sr31* from rye and *Sr44* from *Thinopyrum intermedium*). None of the *Sr* genes provide resistance to all races of stem rust. For instance many of them are ineffective against the Ug99 lineage (Singh *et al.*, 2011). Notably Ug99 has virulence against *Sr31*, which was effective against all previous stem rust races (Singh *et al.*, 2011).

A sequence-tagged-site (STS) marker is reported linked to *Lr28*, a leaf rust resistance gene in wheat. RAPD (random amplified polymorphic DNA) analysis of near-isogenic lines (NILs) of *Lr28* in eight varietal backgrounds was carried out using random primers. Ten microsatellite sequences were found, and three of them were polymorphic in our population and were genetically mapped close to *Lr34*. Therefore, SWM10 is a highly useful marker to assist selection for *Lr34* in breeding programs worldwide (Bossolini *et al.*, 2006). Molecular markers are expected to make an increasing impact on our ability to select gene combinations needed to enhance the durability of

resistance. Advances in molecular plant pathology, however, have made marker assisted selection a routine task. With the help of PCR-based DNA markers, number of leaf and yellow rust, powdery mildew and spot blotch resistance gene(s) can be detected.

Although, many reports of tagging and mapping of several disease resistance genes and QTLs are available in wheat (Langridge *et al.*, 2001) however, not many reports are available for spot blotch. For this disease, the association of resistance with microsatellite markers in bulks of susceptible and resistant progeny lines was reported (Sharma *et al.*, 2007). The QTLs for spot

Table 2: List of markers reported to be linked with leaf rust, stem rust and spot blotch resistance genes/QTLs

Trait	Locus	Marker name	Size (bp)	Chromo-some	Reference
Leaf rust					
	Lr19	GB	198, 180	7D	Prince *et al*., 2001
	Lr21	Ksu- D14	885	1D	Talbert *et al.*,1994
	Lr22a	Xgwm296	121 to 131	2D	Hiebert *et al*., 2007
	Lr25	*Lr25F20/Lr25R19*	1800	4A	Procunier, 1995
	Lr29	*Lr29F18/Lr29R18*	900	4A	Procunier, 1995
	Lr32	Wmc43	346	3D	http://maswhat.ucdavis.edu
		Barc135	273	3D	http://maswhat.ucdavis.edu
	Lr34	csLVLr34	150	7D	Lagudah *et al*., 2009
	Lr39	*GDM35*		2D	http://maswhat.ucdavis.edu
	Lr46	Xgwm259	105	1B	William *et al*., 2003
	Lr47	PCAPSR	394	2A	Helguera *et al*., 2000
	Lr50	Xgwm382	139	2B	http://maswhat.ucdavis.edu
	Lr51	S30-13L AGA7-759R	783	1B	http://maswhat.ucdavis.edu
		GDM87	110	2B	
	Lr67	CFD71	214	7D	http://maswhat.ucdavis.edu
		CFD23	211	7D	
	Lr68	cs7BLNLRR	738	7B	http://maswhat.ucdavis.edu
Stem rust					
	Sr2	*Gwm* 533	120	3B	Hayden *et al*., 2004
		stm598tcac	61		http://maswhat.ucdavis.edu
		stm559gag	85		http://maswhat.ucdavis.edu
		csSr2	172		http://maswhat.ucdavis.edu
	Sr13	Gwm 427 & Wmc 580	293	6A	Simmons *et al*., 2011
	Sr22	Wmc633	170 to 260	7A	Olsen *et al*., 2010
	Sr 26	Sr26#43	207	6A	Mago *et al*., 2005
	Sr 36	STM-773-2	155	2B	Hayden and Sharp, 2001
	SrCad	FSD-RSA	275	6D	Hiebert *et al*., 2011
		cfd 49	180, 212		
	Sr39	Sr39-F2	900	2B	Gold *et al*.,1999
		Sr39#22r	487		Mago *et al*., 2009
Spot blotch					
	QSb.bhu-2A	*Gwm425-Barc159*	180	2A	Kumar *et al*., 2009
	QSb.bhu-2B	*Gwm 148-Barc 91*	184	2B	,,
	QSb.bhu-5B	*Gwm 67-Gwm 213*	120	5B	,,
	QSb.bhu-6D	*Barc 175-Gwm 732*	170	6D	Kumar *et al*., 2010
	QSb.bhu-7D	*Gwm 111-Gwm1168*	210	7D	,,
	Sb1	*Gwm 1220-Swm10*			Lillemo *et al*., 2013

blotch resistance in the Chinese wheat variety, 'Yangmai 6' were mapped on chromosome 2A, 2B, 5B and 6D (Kumar et al. 2009). However, more information with respect to the identification of QTLs in different genetic background was generated when QTLs were mapped in two other resistance sources ('Ning 8201' and 'Chirya 3') and to compare the chromosomal locations of QTLs with 'Yangmai 6' to identify diagnostic markers that can be used for marker assisted selection and to make an effective breeding program (Kumar *et al.*, 2010). List of markers reported to be linked with leaf and stem rust and spot blotch resistance genes/QTLs are given in table 2.

Future strategies

Identification of exploited and unexploited resistant gene(s) for each disease and combine them together for the development of multiple pest resistant variety should be the aim of future breeding programs. For this purpose, resistance genes especially those which can provide resistance against prominent races of economically important diseases and where linked markers are available to be used in marker assisted selection (MAS). Characterization of wheat germplasm at pathological level followed by their molecular characterization through linked markers for known resistance genes to leaf & stem rusts and spot blotch would be one of the best possible approaches to combat with the growing problem of biotic stresses in wheat.

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Research Opinion & Topics

A report of the third term of the National BioResource Project-Wheat, Japan: Fiscal year 2013

Shuhei Nasuda
Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University, Japan
*Corresponding author: Shuhei Nasuda (nasushu@kais.kyoto-u.ac.jp)

I am very glad to write this report for the activities of the third term of National BioResource Project-Wheat (NBRP-Wheat) Japan, because I can conclude that the project has been successfully progressed during the fiscal year 2013. We could provide research materials, namely seed resources and DNA resources, to worldwide users. The propagation of seed resources supervised by Dr. Taihachi Kawahara was in progress as we planed. Deposition of a cosmid library of *Aegiplos tauschii* is a good news for the research community, which is a great contribution made by Drs. Kawaura and Ogihara at Yokohama City University. I deeply thank all the researchers involving in the NBRP-Wheat projects for their tireless devotion to the public services.

We began establishing a system to preserve seed stocks in a unified format, which could not have been realized without additional financial support from the Ministry of Education, Culture, Sports, Science and Technology, Japan. I thank the members of the steering committee of the National BioResource Project for their understanding of the current difficult situation in wheat research community in terms of succession of the seed resources. I would like to especially thank to deceased Professor Dr. Kazuo Moriwaki who continuously encouraged us and preached us the importance of the genetic diversity. In the new system, information of the seed resources kept in two laboratories in Kyoto University will be stored in a unified format of a computer program and we will readily monitor the amount of the stocks. We will continue this unification effort in the fiscal year 2014.

I would like to also mention that NBRP-Wheat has supported a session in the 12th International Wheat Genetics Symposium held in Yokohama during September 8-14. The session was titled as Genomic Assessments of Global Wheat Genetic Resource Collections (Supported by NBRP-Wheat and CIMMYT). We concluded that resource centers should work together to establish a coordinated preservation and utilization of the resource materials. NBRP-Wheat will take part in an expert working group of the genetic resources in Wheat Initiative (http://www.wheatinitiative.org) for coordinated conservation and utilization of wheat resources.

Finally I would like to thank Professor Takashi R. Endo who retired from Kyoto University at the end of March, 2014 after long public service in preservation and distribution of the wheat genetic stocks including his famous deletion stocks. It is Dr. Endo who served as the program manager in the first and second terms of the NBRP-Wheat, and who kept all genetic stocks very clean. I hope we can continue keeping the quality of resources high as he first intended.

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Research Opinion & Topics

The report of National Bioresource Project-Wheat III. Seed resources, 2013.

Taihachi Kawahara [1]*, Takashi R. Endo [2], Shuhei Nasuda [2], Masayuki Isshiki [3] and Tomohiro Ban [3]

[1] Laboratory of Crop Evolution, Graduate School of Agriculture, Kyoto University, Mozume, Muko 617-0001, Japan

[2] Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University, Kitasirakawa-Oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan

[3] Kihara Institute for Biological research, Yokohama City University, Maioka, Totuka-ku, Yokohama 244-0813, Japan

* Corresponding author: Taihachi Kawahara (e-mail:kawahara.taihachi.2z@kyoto-u.ac.jp)

Maintenance of seed resources: Regenerated seeds of total of 1,325 strains were harvested until early summer in 2012. Three hundred and fourteen tetraploid wheat were grown in Kihara Institute for Biological Research, Yokohama City University (KIBR) and 1,011 strains consisting of several wild species and landraces were grown in Graduate School of Agriculture, Kyoto University.

Total of 1,210 strains were sown in autumn 2013, 319 in KIBR and 891 at Graduate School of Agriculture, Kyoto University.

Distribution of seed resources: Total of 849 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		
	Total	Domestic	Overseas
LPGKU	177	51	228
KU (MOZUME)	197	288	485
KT (KIBR)	128	8	136
TACBOW	0	0	0
Total	502	347	849

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

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Research Opinion & Topics

National BioResource Project of Japan III : DNA resource of Wheat, 2013

Kanako Kawaura, Kazuko Umeda, Yasunari Ogihara
Kihara Institute for Biological Research, Yokohama City University, Maioka-cho 641-12, Yokohama 244-0813, Japan
*Corresponding author: Yasunari Ogihara (yogihara@yokohama-cu.ac.jp)

The third phase of the National BioResource Project of Japan "Wheat" has been started in 2012. We, herein, summarize the activity of the second year of the third phase of NBRP-Wheat. DNA resource team 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 gene expression microarray harboring 38K gene probes. The microarray is available from the Agilent Technologies Co. Ltd.

Additionally, TAC library of Chinese Spring wheat has been screened in response to the request. Furthermore, we started providing a cosmid library of *Aegilops tauschii* (KU-2094) containing ca. 250K clones.

In 2013 seasonal activity, 38 cDNA clones requested from four institutions inside of Japan, and 7 clones requested from abroad 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.

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Research Opinion & Topics

"Core-collection" Project of the National BioResource Project-Wheat, Japan: 2013 Progress report

Shotaro Takenaka, Miyuki Nitta, Taihachi Kawahara, Shuhei Nasuda
Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University, Japan
*Corresponding author: Shuhei Nasuda (nasushu@kais.kyoto-u.ac.jp)

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

National Bioresources Project-Wheat (NBRP-Wheat) stores nearly 12,000 accessions of wild species, landraces and historic cultivars of wheat and its relatives (*Triticum* and *Aegilos* species). The main part of the collections derives from the several expeditions that Kyoto University has dispatched 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 purity guaranteed by successive self-pollination. The materials and collection site data are well kept at the Laboratory of Crop Evolution (the former Plant Germplasm Institute), Graduate School of Agriculture, Kyoto University, however, the genetic diversity and phenotypic variation are scarcely recorded. Rapid development of wheat genomics will make our stocks more important than before in basic and applied wheat research activities.

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 summarize the progress in the fiscal year 2013.

Hexaploid wheat core-collection

As reported in last year (Nitta and Nasuda 2013), we could establishe the first version of the hexaploid core collection composed of 192 hexaploid wheat accessions. The detail of the core-collection will be published elsewhere (Takenaka et al., in preparation). The set has genotype information adapting the DArT array technology and basic phenotypic characters. The F_1 and F_2 seeds between the accessions (female parent) and Norin 61 or Chinese Spring wheat (male parent) are available with specific arrangement. Those who want to use the materials, please contact to nasushu@kais.kyoto-u.ac.jp.

Tetraploid wheat core-collection

We genotyped a total of 2,008 AABB and 401 AAGG accessions by the small number of DArT array markers. Based on the genotype data we established a tetraploid wheat core-collection with the 196 accessions representing the genetic diversity. The detail of the genotyping is now in preparation for publication to a scientific journal (takenaka et al., in preparation). We are planning to add marker genotype more densely to the core-collection by the genotyping-by–sequencing approach (Poland et al., 2012). In fall 2013, we started cultivation of the core-collection to propagate seeds. We are planning to pre-release the material to the people who wish to phenotype their interested characters.

Diploid wheat core-collection

We finished DNA preparation for the 501 AA genome species. Those include 50 *T. monococcum* and 38 *T. urartu*, and 413 *T. boeoticum* accessions. We will add some *T. urartu* accessions introduced from USDA. The genomic DNA will be sent to DArT for genotyping with a small number of array-based markers in FY 2014.

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Wheat Inf. Serv. 118: 25-30, 2014. www.shigen.nig.ac.jp/ewis

Meeting Reports

The Ninth Triticeae Meeting of Japan, 2014

Hitoshi Matsunaka
NARO Kyushu Okinawa Agricultural Research Center, 496 Izumi, Chikugo Fukuoka, 833-0041, Japan
Corresponding author: Hitoshi Matsunaka (E-mail: matunaka@affrc.go.jp)

The Ninth Triticeae Meeting of Japan was held at Chikugo research station of NARO Kyushu Okinawa Agricultural Center (NARO/KARC) on April 18 and 19, 2014. About 40 attendee including researchers and students from universities and institutes participated in the meeting. The meeting had 13 oral presentations and 15 poster presentations. The abstracts of oral presentations and poster titles are shown below. Next meeting will be held in Mie University next year.

ABSTRACTS & TITLES

Oral Presentation

O01. The new research project of the ministry of agriculture, forestry, and fisheries of Japan in 2014

Shunsuke Oda
NARO Institute of Crop Science, Japan

The ministry of agriculture, forestry, and fisheries of Japan launched new research project from 2014 to 2018. In that project, wheat and barley breeding program should be carried out according to requirement of milling companies to promote new varieties. Aim of the project is release of three hard wheat varieties and two barley varieties adapted to cold region and temperate region in five years.

O02. Recent advance of the two-rowed barley breeding for high-yielding in Japan

Naoyui Kawada
NARO Kyushu Okinawa Agricultural Research Center, Japan

Japanese two-rowed barley breeding for high-yielding has been extremely successful throughout the last 30 years. First phase of the breeding efforts had be achieved by a development of a food barley cultivar 'Nishinochikara' (released in 1987) and a malting barley cultivar 'Takaho-Golden' (released in 1995), which ware high-yielding (5t/ha in the breeding field) and early maturing, and also had valuable disease resistances (e.g. Barley Yellow Mosaic Virus, powdery mildew and fusarium head bright). In these cross-breeding, high-yield, stiff-culm and resistance to powdery mildew can be derived from an elite European spring barley cultivar 'Mona'.

In the next phase, high-yielding traits like short-straw and heavy-tillering were introduced to 'Nishinochikara' by a cross-breeding with Japanese breeding lines, and a food cultivar 'Nishinohoshi' was selected in 1997. However these high-yielding traits were combined in 'Nishinohoshi', the grain yield was not improved, because of a minor decrease in the thousand kernel weight. In a malting barley, the cross-breeding with Japanese elite malting and food barley lines, combined stiff-culm, heavy grain filling and excellent malting quality, a high-yield (5.5t/ha in the breeding field, ca. 10% superior to 'Takaho-Golden') malting barley 'Sachiho-Golden' was developed in 2005.

Recently, a outstanding high-yield (7t/ha in the breeding field, ca. 30% superior to 'Nishinohoshi') food cultivar 'Haruka-nijo' was developed from the cross between elite lines with heavy-tillering, stiff and short-culm, heavy grain filling and valuable disease resistances. The parent lines involve a short-culm and heavy-tillering cv. 'Triumph' from Europe, superior high-yielding potential can be based in part on a semi-dwarf gene, *sdw1* of 'Triumph'.

Photosynthesis products accumulated in barley grain are derived half from Spike and awn, according to a report of Thorne and Hojyo (1965). And stem and leaf sheath were showed as a critical source of photosynthesis in barley plant (Takeda 1978). Based on these features, cycles of cross-breeding and selection for heavy-tillering and stiff-culm have be carried out consistently, and led to a successful high-yielding barley.

O03. Wheat breeding in Kyushu Okinawa

Agricultural Research Center

Kazuhiro Nakamura
NARO Kyushu Okinawa Agricultural Research Center, Japan

Wheat breeding for Kyushu district has been carried out since 1932 in Chikugo city. Cultivation area of wheat in Kyushu was about 32,400ha (2009-2010 season). The wheat cultivation area of Kyushu occupies 17% of the whole Japan. The wheat varieties which Kyushu Okinawa Agricultural Research Center has registered are cultivated in more than 95% of the wheat cultivation area of Kyushu. 'Shiroganekomugi' is the most cultivated in Kyushu, which is a soft wheat for Japanese noodle or confectionery. The second one is 'Chikugoizumi', which is a soft wheat and partial waxy wheat. 'Minaminokaori' is a hard wheat for bread, which is cultivated 3,600ha in southwestern Japan. 'Chikugomaru' released in 2012 showed the lowest accumulation of deoxynivalenol among Japanese wheat cultivars. Besides high yielding and scab resistance, one of our main breeding objectives is new demanded and local specialty wheat. 'Nagasaki W2' was developed in collaboration with Nagasaki Prefecture for Champon noodles use which is a local cooking of Nagasaki.

O04. DNA marker development for barley grain hardness and endosperm component selection

Asuka Takahashi
NARO Western Region Agricultural Research Center, Japan

Grain hardness and β-glucan content are the most important quality characteristics of pearled barley. Barley seed proteins Hordoindolines are homologs of wheat Puroindolines, which are associated with grain hardness. Two *Hordoindoline* (*Hin*) genes are present, namely *Hina* and *Hinb*; *Hinb* further consists of two genes, *Hinb-1* and *Hinb-2*. A *Hinb-2* allele-specific DNA marker was developed to distinguish between the wild type (*Hinb-2a*) and null (*Hinb-2b*). Analysis of grain hardness among F2 lines showed significantly higher grain hardness in the null lines. Further, in the *Hinb-2* null lines, the pearling time and grain grassiness were high, and broken kernel ratio was low.

Barley lines with *high amylose* (*amo1*) gene showed high β-glucan contents. We were able to develop DNA markers linked to *amo1* at the neighboring regions. Subsequently, barley lines with high β-glucan content were selected from the experimental F2 lines by using the DNA marker.

O05. Status and prospects of DNA markers for starch properties in wheat and barley

Mika Saito
NARO Tohoku Agricultural Research Center, Japan

Abstract not available.

O06. Distribution of photoperiod-insensitive alleles *Ppd-A1a*, *Ppd-B1a* and *Ppd-D1a* in Japanese wheat cultivars

Masako Seki
NARO Agricultural Research Center, Japan

Heading time of wheat is a complex characteristic controlled by narrow-sense earliness and is modified by vernalization response and photoperiod response. It has been also reported that the photoperiod response is the major determinant of earliness of autumn-sown wheat in central and southwestern Japan (Tanio *et al*. 2006, Yasuda and Shimoyama 1965, Yoshida *et al*. 1983). In this study, the genotype of photoperiod response genes *Ppd-A1*, *Ppd-B1* and *Ppd-D1* in 240 Japanese wheat cultivars were determined using a PCR-based method (Beales *et al*. 2007, Nishida *et al*. 2013). Most Tohoku-Kyushu cultivars (97.5%) carried *Ppd-D1a*; and extra-early cultivars (5.0%) carried the two photoperiod-insensitive alleles *Ppd-B1a* and *Ppd-D1a*. However, no cultivars carried *Ppd-A1a*. Pedigree analysis of extra-early wheat cultivars showed that *Ppd-B1a* in three extra-early commercial cultivars was inherited from 'Shiroboro 21' by early-heading Chugoku lines bred at the Chugoku Agriculture Experimental Station. In Japan, except in Hokkaido, the rainy season starts before the wheat harvest; thus, early cultivars with *Ppd-D1a* have been selected to avoid damages such as preharvest sprouting and Fusarium head blight. Furthermore, it is suggested that the introduction of the *Ppd-B1a* accelerated the early-maturity wheat breeding in Japan. In contrast, among Hokkaido cultivars, none of the cultivars carried *Ppd-B1a*, and the frequency of the *Ppd-A1a* and *Ppd-D1a* alleles generally differed between winter wheat and spring wheat. Among winter wheat cultivars, 41.4% and 24.1% carried *Ppd-A1a* and *Ppd-D1a*, respectively. In contrast, in spring wheat cultivars, most (90%) did not carry photoperiod-insensitive alleles. Pedigree analysis of Hokkaido winter wheat cultivars showed that 'Purple Straw' and 'Tohoku 118' were one of the donor(s) of *Ppd-A1a* and *Ppd-D1a* in Hokkaido wheat cultivars, respectively. Wheat cultivars recently developed in Hokkaido carry photoperiod-insensitive alleles at a high frequency. For efficient utilization of *Ppd-1* alleles in the Hokkaido wheat-breeding program, the effect of

Ppd-1 on growth pattern and grain yield should be investigated.

O07. Fluctuation of flowering time and its genetic control in Japanese barley under warm winter condition

Hidetaka Nishida[1], Takuma Kaneko[1], Masahiro Tsuchiya[1], Emiko Aoki[2], Hitoshi Matsunaka[3], Masaya Fujita[2, 3], Masato Taira[2], Takashi Yanagisawa[2], Kenji Kato[1]
[1]Graduate School of Environmental and Life Science, Okayama University, Japan
[2] NARO Institute of Crop Science, Japan
[3] NARO Kyushu Okinawa Agricultural Research Center, Japan

In south-western part of Japan, early-flowering varieties of barley have been developed to avoid preharvest sprouting and Fusarium head blight, both of which occur when spikes at maturing stage are hit by intermittent rains during rainy season around June and July. However, such varieties, when exposed to warm winter caused by recent global climate change, develop reproductive shoot apex much earlier than usual years, resulting in decrease in number of tillers, biomass, and yield. In addition, reproductive shoot apex also suffers from frost injury by untimely chilling temperature in early spring. To avoid these problems, it will be necessary to develop early-flowering varieties whose flowering is not promoted very much even under warm winter conditions. However, genetic basis of such character still remains unknown. In this study, we reported on 1) flowering-related (vernalization and photoperiod sensitivity) genes that contributed to early flowering of Japanese varieties, 2) flowering-related genes that do not promote development of reproductive shoot apex very much even under warm winter conditions, and 3) effect of these genes under three different conditions (Tsukuba, Okayama, and Chikugo) where temperatures during winter are different.

O08 Assessment of the *Aegilops tauschii* biodiversity as genetic resources in common wheat breeding; a case study in alteration of heading time

Shigeo Takumi
Graduate School of Agricultural Science, Kobe University, Japan

Aegilops tauschii Coss., a wild diploid progenitor of allohexaploid wheat, is a important genetic resource for breeding of the D genome in common wheat. The genetic variation in the D genome of common wheat is less than those in the A and B genomes, which is considered to be due to limited contribution of the *Ae. tauschii* population for hexaploid wheat evolution. Therefore, natural variation in the *Ae. tauschii* populations offers potential for improving modern varieties of common wheat. We have produced numerous synthetic hexaploid wheat lines through crosses of a tetraploid wheat cultivar Langdon and *Ae. tauschii* accessions. Common garden experiment with these synthetic wheat lines showed that they exhibited wide variation in the flowering-related traits, and that the large variation in heading time observed in *Ae. tauschii* is also present in the hexaploid synthetics (Kajimura et al. 2011). To elucidate the genetic basis of variation in flowering-related traits, we analyzed quantitative trait loci (QTL) affecting time to heading, flowering and maturity, and the grain-filling period using four different F_2 populations of synthetic hexaploid wheat lines. In total, 10 QTLs located on six D-genome chromosomes (all except 4D) were detected for the analyzed traits (Nguyen et al. 2013). These QTL are effectively expressed in the hexaploid wheat genetic background of synthetic wheat. To assess the effects of the identified QTLs in the Japanese common wheat cultivar, an early heading line of wheat synthetics was crossed to a cultivar Kitanokaori, and QTL analysis for flowering-related traits was conducted using the F_2 population between a synthetic wheat line and a common wheat cultivar. Only two major QTLs were significantly found at the *Ppd-D1* and *Vrn-A1* loci, and a *Ppd-D1* allele from Kitanokaori and a *Vrn-A1* allele from Langdon mainly contributed to early heading/flowering time. No other QTLs for flowering-related traits were detected in the synthetic wheat/Kitanokaori F_2 population due to the strong effect of *Ppd-D1*. In the homozygous individuals with the late flowering allele of *Ppd-D1*, however, the early lowering alleles derived from the early heading line of wheat synthetics significantly contributed to the early heading/flowering phenotype. Therefore, these studies demonstrate that synthetic wheat lines can be useful for the identification of new, agriculturally important loci that can be transferred to hexaploid wheat.

O09. Identification of a novel earliness gene in wheat using barley genome information

Nobuyuki Mizuno[1], Miyuki Nitta[1], Kazuhiro Sato[2], Shuhei Nasuda[1]
[1]Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University, Japan
[2] Institute of Plant Science and Resources, Okayama University, Japan

An X-ray mutant showing an early flowering phenotype has been long recognized in einkorn wheat (*Triticum monococcum* L.), for which a major QTL for heading time has been identified on

the long arm of chromosome 3A. In the presentation, we demonstrated that recently advanced genome information of barley helped to develop the molecular markers on the target region utilizing highly conserved synteny between wheat and barley genomes. Fine-mapping using 14 genic markers revealed that an ortholog of the *LUX ARRHYTHMO* (*LUX*)/*PHYTOCLOCK 1* (*PCL1*) gene found in *Arabidopsis* was tightly linked to the early heading phenotype and deleted in the einkorn mutant. The einkorn wheat mutant showed distorted gene expression patterns of clock-genes and significant up-regulation of photoperiod-response related genes such as *Ppd-1* and two wheat *CONSTANS*-like genes. In addition, the transcript accumulation level of the wheat florigen gene (*WFT*) was higher than that in wild type. These results strongly suggested that the wheat *PCL1* homologue (*WPCL1*) is the most likely candidate gene for controlling the early heading in the early mutant.

O10. Analyses of population structure and genetic diversity of the NBPR-Wheat core-collections

Shotaro Takenaka[1], Miyuki Nitta[1], Taihachi Kawahara[2], Shuhei Nasuda[1]
[1]Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University, Japan
[2] Laboratory of Crop Evolution, Graduate School of Agriculture, Kyoto University, Japan

One hundred and ninety accessions were selected as National BioResource Project (NBRP) -Wheat hexaploid wheat core-collection from ca. 3,500 accessions based on their taxonomy and geographical distributions. The genetic diversity was investigated by 1,504 DArT markers, whose locations on linkage maps are known. We showed that our hexaploid core-collection can be divided into three groups (Group-α, β, γ) based on the results of the model-based clustering analyses. Group-α mainly consisted of Asian accessions. Group-γ was composed of European accessions including most of *T. spelta*. Group-β was an admixed group between Group-α and Group-γ, which is consisted of accessions derived from various regions and including most of modern cultivars. When we analyzed population structures within each more in detail, geographically close accessions formed clusters although they are taxonomically classified as different species. These results indicated that genetic diversity of hexaploid wheat has been maintained within geographically isolated populations and classification of hexaploid wheat accessions does not reflect biological species. Then we did association analyses for eighteen phenotypes (heading time, spike length, 100-grain weight *etc.*). We could detect, for example, some MTAs (marker-trait associations) related to the 100-grain weight on chromosome 3B, 5B and 7A at a significance level of 1% after Bonferroni multiple test correction.

NBRP-Wheat stores ca. 2,300 tetraploid wheat accessions including emmer wheat (AABB) and timopheevii wheat (AAGG). The genetic diversity was assayed by 300 DArT markers. We found that emmer wheat accessions can be divided into three groups (AB-g1, -g2, -g3) based on the results of the model-based clustering analyses. AB-g1 was an admixed group between wild emmer (*Triticum dicoccoides*) and hulled emmer (*T. dicoccum etc.*). AB-g2 mainly consisted of Ethiopian free-threshing emmer (*T. durum etc.*). AB-g3 was composed free-threshing emmer from around the world excluding Ethiopia. Timopheevii wheat accessions were divided further into two clusters (AG-g1, -g2) based on the model-based clustering analyses. AG-g1 consisted of wild timopheevii wheat (*T. araraticum*) from Turkey to Iraq. AG-g2 consisted of wild timopheevii wheat (*T. araraticum*) from Southwestern Turkey to Syria. Domesticated timopheevii wheat accessions (*T. timopheevii*) were admixture evenly contributed by AG-g1 and AG-g2.

One hundred ninety two accessions were selected as NBRP-Wheat tetraploid wheat core-collection so as to cover the genetic diversity of the entire collection. We found more than 50,000 SNPs from NBRP-Wheat tetraploid wheat core-collection by the Genotyping by Sequencing approach.

O11. Grain dormancy level of proanthocyanidin-free barley mutants

Eiko Himi
Institute of Plant Science and Resources, Okayama University

The grain color of wheat is an important trait because it affects not only end-product quality but also grain dormancy. As compared to white-grained lines, red-grained lines show higher dormancy and are more tolerant to preharvest sprouting. The grain color is controlled by the *R* gene, which encodes the Myb-type transcription factor, Tamyb10. Tamyb10 regulates the expression of genes involved in a proanthocyanidin synthetic pathway, such as the *dihydroflavonol 4-reductase* (*DFR*) gene.
We have already isolated three *DFR* genes of wheat, and we found that they are located on chromosomes 3A, 3B, and 3D, respectively. If these three *DFR* genes were all mutated, the line would have white grains. However, no mutants of *DFR* genes were found in the wheat.

On the other hand, in barley, a number of

anthocyanin and/or proanthocyanidin-free mutants have been generated because proanthocyanidins in grain result in beer haze and discoloration after cooking. The anthocyanin/proanthocyanidin-free barley mutants are called *ant* mutants. *Ant* loci are classified into *Ant1* to *Ant30*; however, only a few loci have been isolated (e.g., *Ant18* encodes DFR). We found that *Ant28* encodes an Myb-type transcription factor, Hvmyb10, and that it is an orthologous gene of *Tamyb10*.

We compared the level of dormancy of an original cultivar (*Ant18/Ant28*), three *ant18* mutants (*ant18/Ant28*), and an *ant28* mutant (*Ant18/ant28*). While the *ant28* mutant showed a lower dormancy level than the original cultivar, the dormancy levels of *ant18* mutants were the same as the original cultivar. Similar tendencies were observed with other *ant* mutants (*ant17* and *ant26*), although these *Ant17* and *Ant26* loci were not identified. These results suggested that the syntenic genes, the *R* gene of wheat and the *Ant28* gene of barley, may have the important role of controlling both grain color and grain dormancy. Therefore, mutation of the syntenic genes may lead to white grains, but it causes a weaker level of grain dormancy.

O12. **Mutation in the ABA catabolic gene reduced germination in wheat**

Makiko Chono
NARO Institute of Crop Science, Japan

The catabolism of abscisic acid (ABA) through ABA 8'-hydroxyase (ABA8'OH) plays an important role in reducing ABA content in seed during imbibition, and thus in promoting germination. To investigate the effect of reduced ABA catabolism on germination in hexaploid wheat, we cloned ABA8'OH homologue (*TaABA8'OH1*) and screened for mutations that lead to reduced ABA catabolism. In a screen for natural variation, one insertion mutation in *TaABA8'OH1-D*, resulting in the change of the amino acid residues conserved between ABA8'OHs in plant, was identified in several Japanese cultivars. We employed one cultivar with *TaABA8'OH1-D* mutation, 'Tamaizumi', to produce a double mutant in *TaABA8'ox1*s using a gamma-radiation treatment. In a screen for induced mutation, one deletion mutant lacking the entire *TaABA8'OH1-A* was identified. A double mutant in *TaABA8'OH1-A*/*TaABA8'OH1-D* showed a reduced expression of *TaABA8'OH1*s and an increased level of ABA content in the embryos during seed maturation and imbibition, and a reduced seed germination in comparison to 'Tamaizumi', a single mutant in *TaABA8'OH1-D*. These results indicated that the reduced ABA catabolism through the mutations in *TaABA8'OH1*s might have a positive effect on the inhibition of germination in wheat.

O13. Development of mutant panel generated by heavy-ion beam irradiation and flowering-time mutants in einkorn wheat

Koji Murai
Department of Bioscience, Fukui Prefectural University, Japan

A large-scale mutant panel of diploid einkorn wheat (*Triticum monococcum*) using heavy-ion beam mutagenesis has been developed, and the flowering-time mutants were systematically screened. Here, I focus on the recently identified four extra early-flowering mutants, named *extra early-flowering1* (*exe1*), *exe2*, *exe3*, and *exe4*, derived from *T. monococcum* strain KU-104-1. Based on the flowering phenotype, four *exe* mutants were classified into two groups, Type I (moderately extra early-flowering type; *exe1* and *exe3*) and Type II (extremely extra early-flowering type; *exe2* and *exe4*). Flowering phenotype of Type II was analyzed in detail in a growth chamber. In comparison with the wild-type (WT) strain KU104-1, the leaf unfolding speed in Type II *exes* were accelerated after 4-leaf stage under both long day (LD) and short day (SD) conditions. Furthermore, the Type II *exes* showed the decreased photoperiod sensitivity. Then, we examined the expression levels of *VRNALIZATION 1* (*VRN1*), a flowering promoter gene, and *Wheat FLOWERING LOCUS T* (*WFT*), a florigen gene, by real-time PCR analysis. The expression revels of *VRN1* and *WFT* in *exes* were increased at earlier stages than in WT under LD condition. Under SD conditions, *VRN1* was highly expressed in Type II *exes*. The level of *WFT* expression was also much higher in Type II *exes* than WT under SD condition. These results indicate that high expression levels of *VRN1* and *WFT* under LD and SD conditions are causes of the extremely extra early-flowering phenotype in Type II *exe* mutants.

<u>Poster Presentation</u>

P01.
Development of DNA markers for genomics-assisted breeding of barley by RNA-seq analysis
Sato, K. (Institute of Plant Science and Resources, Okayama Univ.)

P02.
NBRP Barley - A model plant for study of environmental stress responses –
Hisano, H. and K. Sato (Institute of Plant Science and Resources, Okayama Univ.)

P03.
DArTseq mapping of the earliness gene in the wheat breeding line "Chogokuwase"
Yamashita, H.[1], M. Takeguchi[1], N. Mizuno[2], M. Nitta[2], H. Nishida[1], M. Fujita[3], S. Nasuda[2] and K. Kato[1] ([1]Grad. Sch. Environ. Life Sci., Okayama Univ, [2]Grad. Sch. Agr., Kyoto Univ., [3]NARO/NICS)

P04.
A hypothesis about the threshold of *VRN1* expression level for flowering competency in wheat
Kitagawa, S and K. Murai (Fukui Prefectural Univ.)

P05.
ABCDE model of bread wheat revealed by the homoeolog-specific expression patterns of MADS-box homeotic genes
Tanaka, M. and K. Murai (Fukui Prefectural Univ.)

P06.
Genome cross-talk regulation of the *VRN1* expression in bread wheat
Yamakage, Y. and K. Murai (Fukui Prefectural Univ.)

P07.
Recombinant inbred lines between early-heading and late-heading synthetic hexaploid wheats produced by crossing of durum wheat cultivar Langdon with *Aegilops tauschii*; Toward identifying the regulator of *VRN1* expression
Isaka, H. and K. Murai (Fukui Prefectural Univ.)

P08.
Identification of genes for Polycomb complex in wheat; Toward understanding epigenetic regulation of *VRN1* expression
Umekita, K. and K. Murai (Fukui Prefectural Univ.)

P09.
Effect of *Aegilops geniculate* cytoplasm on flowering in wheat; Toward verifying mitochondrial vernalization sensor hypothesis
Narita, K. and K. Murai (Fukui Prefectural Univ.)

P10.
Overview and research progress of SATREPS Afghan wheat project
Manickavelu, A.[1], M. E. Haque[1], H. Tsujimoto[2], M. Matsui[3], Y. Kondo[4] and T. Ban[1] ([1]KIBR, Yokohama City Univ., [2]ALRC, Tottori Univ. [3]RIKEN BMEP, [4]Kanto Gakuin Univ.)

P11.
Establishment of methodology for root study under drought condition for screening the Afgan wheat landraces
Osmani, A. A., A. Manickavelu, M. E. Haque and T. Ban (KIBR, Yokohama City Univ.)

P12.
Characterization of Afgan wheat landraces for strip rust and growth habit
Stanikzai, A. S., A. Manickavelu, M. E. Haque and T. Ban (KIBR, Yokohama City Univ.)

P13.
Study of Afghan wheat landraces to identify new ediotypes for high yielding potential
Maqsodi, A. M., M. E. Haque, A. Manickavelu and T. Ban (KIBR, Yokohama City Univ.)

P14.
Identification of root ediotype for Afganistan rain fed wheat cultivation
Ahmadi, S. H., A. Manickavelu, M. E. Haque and T. Ban (KIBR, Yokohama City Univ.)

P15.
Study Afghan wheat landraces for alkali tolerance
Zaheri, E. M., A. Manickavelu, M. E. Haque and T. Ban (KIBR, Yokohama City Univ.)

(Editorial comment)
Dr. H. Matsunaka summarized the meeting of “The Ninth Triticeae Meeting of Japan, 2014” held on April 18 and 19 in NARO/KARC, Japan. We circulate the abstracts of oral presentations edited by K. Kawaura.

Wheat Inf. Serv. 118: 31-32, 2014. www.shigen.nig.ac.jp/ewis

Others

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

Journal articles:

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

Book chapters:

Peacock WJ, Dennis ES, Gerlach WJ (1981) Molecular aspects of wheat evolution: repeated DNA sequences. In: Evans LT and Peacock WJ (eds.) Wheat Science -

Today and Tomorrow. Cambridge Univ. Press, Cambridge, UK, pp. 41-60.

Books:

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

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

Abbreviations

Abbreviations should be explained at first occurrence.

Symbols and Units

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

Nomenclature

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

Nucleotide sequences

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

Tables

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

Figures

Figures must be numbered consecutively. Prepare a separate file for each figure.

Outline of the publication process

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

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

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

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