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ITEMS FROM PAKISTAN

COMSATS INSTITUTE OF INFORMATION TECHNOLOGY
Department of Environmental Sciences, CIIT, Abbottabad, Pakistan.

Wheat-improvement program: Overall goals.

M. Maroof Shah, Mustafa S. Nawaz, A. Hassan, I. A. Raja, B. Nawab, A. Pervez, K. Maqbool, and S. Khan.

One of the major areas of concentration in research and development in the department of Environmental Sciences at COMSATS, Abbottabad, is the application of biotechnology in plants and environment. Work is in progress to manipulate genetic mechanisms underpinning biotic and abiotic environmental stresses, quality traits, and energy potential in a number of important plant species. Wheat, among the prime crops of Pakistan nourishing millions every day, is facing serious problems of low yield, poor adaptation, disease susceptibility, and abiotic stress. A lack of genetic understanding and complexity of the genome are the most important factors. The main objectives of our wheat-improvement program at COMSATS, Abbottabad, are to target the genetic basis of each problem using conventional and advanced techniques. Our immediate objectives are to explore the genetic diversity in the available germ plasm using conventional and molecular markers. Comparative genomics and association mapping were considered bigger potential tools for understanding and underpinning biotic and abiotic environmental stresses, quality, bio-fuels, and yield-related traits. Introducing and introgressing genes from wild relatives by understanding chromosome-pairing mechanisms, targeting specific homoeologous groups and chromosome bins or gene-rich regions, and transformation are some of the focus areas.

Targeting the Ph1 gene for wheat improvement.

M. Maroof Shah, A. Pervez, and Ummara W. Khan.

Pakistan has unique wealth of wild wheat germ plasm that can be used to transfer useful genes into cultivated wheat to broaden its gene pool. However, the genetic activity of the gene 'pairing homoeologous' (*Ph1*), the chromosomes of wild relatives either do not pair or very poorly recombine with wheat, resulting in either no transfer or transferring of large segments of chromosomes with undesirable blocks of genes from the alien species. One option is to utilize deletion based mutant lines for *Ph1* locus available in Chinese spring wheat for transferring the alien genes into cultivated wheat. The main difficulties during such alien gene transfer experiments are scoring of the plants for the presence or absence of

Ph1 gene segment. With the advent of molecular marker techniques, the chromosomal region containing *Ph1* gene may be enriched and the identified linked markers will provide a direct and swift mean to score the gene where alien transfer will be employed. Saturation mapping in the *Ph1* gene region will lead to eventually clone the gene which will have tremendous impact on all of the biological systems in living species. In current study we aimed to identify molecular markers using comparative genomic approaches to enrich the *Ph1* gene region. We have used genetic linkage maps and physical maps in wheat and other cereals and have identified more than 30 markers potentially lying in 0.5L region that contains the *Ph1* gene region. Among these 18 seems to be specific for *Ph1* region (Fig. 1). The *Ph1* gene region markers identified by this approach were observed for their genetic position on the chromosome of cereal crops collinear to wheat. The markers will be tested either using restriction based DNA analyses or amplification based techniques by generating site specific primers. This study will have global impact in wheat genetics and breeding where the geneticists and breeders will be able to transfer a wealth of genes from the alien sources expanding the wheat gene pool for yield and quality traits.

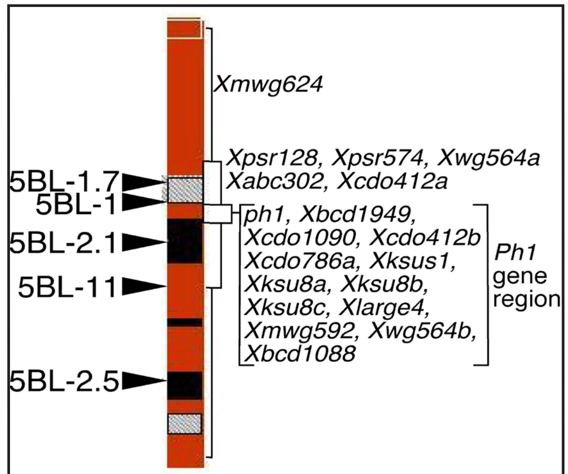

Fig. 1. Physical map of the 5L0.5 gene-rich region containing the *Ph1* locus.

Fine manipulation of the gene even without its cloning may assist in transferring alien genes into wheat. The greater challenges are to score the gene in the lines and populations that carries its null alleles. The phenotypes of the chromosomal genotypes are not manifested in the observable traits in the greenhouse or the field. The known phenotype, in this case, is chromosome-pairing (multivalent), which is a long and laborious task. The current study may serve as a means to score the gene by identifying closely linked molecular marker (s) to the gene region. The information will be utilized to improve wheat cultivars in Pakistan by transferring useful genes from its wild relatives using the *Ph1* gene systems and the linked markers.

Recombinant chromosome lines (RCLs) of wheat chromosome 3A.

Mohammad M. Shah, K. Maqbool, S.H. Shah [1], M. Ali [1], and P.S. Baenziger [2].

[1] Institute of Biotechnology and Genetic Engineering, NWFP, Peshawar, Pakistan, and [3] Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583, USA.

Recombinant chromosome lines for wheat chromosome 3A were developed between winter wheat cultivar Cheyenne (CNN) and a chromosome substitution line DS CNN–WI (3A) at the University of Nebraska, Lincoln, USA (Morris et al. 1960-84) and characterized for QTL mapping and gene identification (Shah et al. 1999). This material is extremely unique; taking more than 30 years of research at the University of Nebraska-Lincoln, U.S. This material is strictly winter habit and needs at least 6 weeks of vernalization to flower and set seed. The number and linkage relationship of the gene(s) or QTL controlling important agronomic traits on chromosome 3A were estimated using DNA markers on RCLs in diverse environments (Shah et al. 1999). The RFLP map developed for chromosome 3A was used to identify and map genes controlling important traits including earliness per se and yield (Shah et al. 1999). The map was reproduced and placed as the first interactive QTL map of wheat chromosomes on the GrainGenes web site by the USDA under 'gene mapathon' http://wheat.pw.usda.gov/ggpages/ggmapathon.html.

The genetic material, including 50 RCLs of chromosome 3A with four winter wheat parental lines, were kindly provided by Dr. P.S. Baenziger, University of Nebraska-Lincoln. The aim of current study was to identify and map QTL responsible for growth habit and plant morphology, because these traits play important role in contributing toward biomass and the final yield. Another important objective was to increase pure seed in a different environment from this precious material, which will be needed for future studies. Confirming early maturity (action of possibly *Eps* gene) was also an interest so that the material can be multiplied and be introduced in the winter and may be semiwinter growing areas of Pakistan where a wheat–maize overlap causes the sacrifice of the grain of one of the crops. Interesting findings were obtained. Without giving the required vernalization treatment, a large amount of seed from a single seed of each of the 50 lines and parental cultivars was obtained. The seed was threshed by hand and carefully stored in the laboratory. Complete data on all the relevant traits were collected and is being analyzed. The means of some of the traits are

Table 1. Means of various qualitative and quantitative traits for 50 recombinant chromosome lines and their winter wheat parental lines grown in Peshawar and Abbottabad, Pakistan, during 2006–07.

Line	Crown morphology	Tiller number	Plant height (cm)	Awn length (cm)	Spike length (cm)	Florets/ spike	Glume length (cm)	Lemma (cm)	Palea (xm)	Spikes/ plant	Seed/ spike
CNN	4	16	86	6.00	10.00	12.33	0.7	0.5	1	10	70
WI	5	24	78	6.33	7.33	15.67	0.6	0.8	0.6	9	97
CNN(WI3A)	7	25	75	8.33	7.33	19.67	1.0	0.7	0.6	14	103
CCWI3A01	4	21	85	6.67	6.67	17.00	0.9	0.8	0.9	8	87
CCWI3A02	4	17	80	7.83	9.00	15.67	0.8	0.9	0.8	9	67
CCWI3A03	7	25	74	6.00	8.33	11.33	0.7	1.0	0.7	9	98
CCWI3A04	5	16	85	8.17	6.17	17.33	0.5	1.0	0.6	10	100
CCWI3A05	5	16	78	6.83	6.33	20.67	0.7	1.0	0.6	6	58
CCWI3A06	8	19	75	5.67	9.33	13.67	0.9	0.6	0.7	8	88
CCWI3A07	5	25	85	5.5	7.00	14.67	1.0	0.8	0.8	9	101
CCWI3A08	6	25	80	8.33	8.83	20.00	0.5	0.9	0.9	6	57
CCWI3A09	9	27	79	7.33	8.33	11.67	0.8	0.7	0.8	6	78
CCWI3A10	7	25	70	7.00	8.50	16.67	1.0	1.0	1.0	10	115
CCWI3A12	4	18	74	6.50	9.00	15.33	0.9	0.6	0.5	8	27
CCWI3A13	6	17	80	7.83	7.17	15.00	0.5	0.8	0.6	10	100
CCWI3A17	5	24	78	8.00	10.33	20.67	0.7	0.8	0.9	8	97
CCWI3A18	5	18	78	8.50	7.33	13.00	0.6	0.8	0.7	14	108
CCWI3A19	8	22	85	8.00	7.50	13.67	0.5	0.7	0.8	14	89
CCWI3A20	3	22	90	7.00	7.50	17.33	0.9	0.8	0.7	12	163
CCWI3A21	7	19	75	5.67	8.33	15.67	0.8	0.8	1.0	17	243
CCWI3A22	3	18	74	6.17	10.17	17.33	0.6	0.6	0.7	16	289
CCWI3A23	8	22	77	5.50	8.67	15.33	0.8	0.8	0.7	10	110
CCWI3A24	4	24	85	8.33	9.33	19.67	1.0	0.6	0.8	12	98
CCWI3A25	8	21	88	8.67	7.83	17.00	0.9	1.0	1.0	17	346
CCWI3A26	3	20	90	8.33	8.50	15.33	0.9	0.5	0.5	13	170
CCWI3A27	4	16	80	6.17	8.50	19.67	0.5	0.8	0.5	9	167
CCWI3A28	9	19	74	5.50	8.50	17.00	0.7	0.9	0.6	12	200
CCWI3A29	7	19	85	6.67	7.83	16.00	0.8	1.0	0.9	17	256
CCWI3A30	9	14	78	8.17	7.33	15.67	1.0	0.9	0.7	19	332
CCWI3A31	7	22	80	6.50	8.00	20.33	0.9	0.5	0.8	7	66
CCWI3A32	4	15	90	8.33	8.33	18.67	0.6	0.7	0.6	7	140
CCWI3A33	6	22	74	5.67	8.50	17.33	0.6	0.8	0.5	15	220
CCWI3A34	4	14	90	7.50	8.50	15.67	0.7	0.9	0.8	8	172
CCWI3A35	4	15	75	8.17	7.33	15.00	0.9	1.0	0.8	15	229
CCWI3A36	7	25	78	7.33	7.33	17.67	0.5	0.8	0.6	7	113
CCWI3A37	8	21	80	7.17	9.50	20.00	0.8	0.8	0.5	11	273
CCWI3A38	9	18	85	5.50	10.17	19.67	0.9	0.6	0.8	20	182
CCWI3A39	9	19	74	6.67	8.67	13.00	1.0	0.7	0.9	17	220
CCWI3A40	8	15	80	8.00	7.67	15.00	0.9	0.8	0.9	11	266
CCWI3A41	7	23	75	8.50	8.17	15.67	0.5	0.8	1.0	17	77
CCWI3A42	8	15	80	6.50	8.00	14.00	0.6	0.7	0.7	9	215
CCWI3A43	9	22	85	7.67	9.00	15.67	0.9	1.0	1.0	10	165
CCWI3A44	6	19	80	7.50	8.17	18.67	0.6	0.7	0.6	11	265
CCWI3A45	8	15	84	7.00	8.50	18.33	1.0	1.0	0.9	17	185
CCWI3A46	3	26	78	8.17	8.17	20.00	0.6	1.0	0.5	13	201
CCWI3A47	7	21	75	7.50	7.00	14.67	0.6	0.6	0.7	10	88
CCWI3A48	8	17	80	8.67	9.50	15.00	0.6	0.6	0.8	12	63
CCWI3A49	8	23	74	6.17	6.67	10.67	0.7	0.7	0.7	14	153
CCWI3A50	9	22	90	7.33	9.00	19.00	0.9	1.0	1.0	14	242
Aapahoe	8	27	78	8.67	7.50	19.00	0.7	1.0	1.0	6	57
Pronghorn	3	15	80	7.83	7.33	19.67	0.7	0.5	1.0	17	300
Millenium	6	19	90	6.33	10.00	15.33	0.9	0.7	0.5	13	162
Alliance	5	27	75	7.17	7.00	12.67	0.9	0.6	0.9	9	176

presented in Table 1 (p. 98). The traits include crown morphology, tiller number, plant height, awn length, spike length, florets/spike, glumes length, seed/plant, spikes/plant, palea, and lemma.

A future goal of this research is to screen the germ plasm for the same traits in different environments and use polymorphic molecular markers on the 3A. Using bin-mapping information and ESTs on physical maps, we may be able to target specific genes in Pakistani wheat germ plasm that will be useful for this crop improvement. Indigenous and overseas collaborations will be sought out. Dr. P. S. Baenziger's wheat program and the John Innes Center, UK, are the potential overseas collaborators in these efforts.

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Wheat regeneration, transformation, and tissue-culture studies.

A. Hassan, S.H. Shah, U.W. Khan, H. Kiran, and M.M. Shah.

Successful callus culture and regeneration is fundamental to transformation of genes across species using genetic-engineering tools. A number of genes were identified and cloned in the cereals and Poaceae that need to be transformed into cultivated wheat. Wheat, being a monocot species, is notorious in regeneration through tissue culture, thus buffering the possibilities of quick gene transformation that may help in its improvement. Although wheat has a poor response to callus production and organogenesis, sustained efforts should continue to tap the benefits of genetic engineering. Pakistan has a wealth of indigenous wheat germ plasm including improved cultivars and land races. Several diploid and tetraploid wheat relatives are spread all over northern Pakistan. After successful establishment of tissue culture facilities in the Department of Environmental sciences, COMSATS University, Abbottabad, a team of scientists has developed an effort on wheat and potato transformation. The key goals for wheat transformation will be disease and stress (mainly drought and salinity) resistance. As part of this effort, plant pathologist Dr. Amjad Hassan, with a team of dedicated students and researchers will lead the work on wheat transformation protocols after optimizing potato tissue culture work. Initially, we will look at optimizing the culture conditions for the regeneration of Bobwhite or Pavon, the main wheat cultivars used in transformation. We have plans to survey our spring and winter wheat germ plasm to find better candidate(s) with maximum regeneration. We also will screen winter wheat substitution lines or CS nulli-tetrasomic lines to identify chromosomal locations of gene(s) responsible for tissue culture response, as well. A majority of the local germ plasm (about 100) and winter wheat cultivars along with chromosomes substitution lines have been collected and screening using different media are underway.

Personnel.

Dr. Mustafa N. Shafqat, a graduate of Kansas State University and researcher in soil sciences and wheat genetics at the University of Washington, Pullman, joined COMSATS University under the Higher Education Commission of Pakistan as Assistant Professor. He joined the wheat project and will work on association mapping of stress-related genes. Dr. Amjad Hassan, a graduate and then Assistant Professor at Niigata University, Japan, in Biosphere Sciences and Molecular Plant Pathology, joined COMSATS University under the Higher Education Commission of Pakistan as Assistant Professor. He will lead wheat tissue culture, transformation, and disease related gene studies. Ms. Ummara W. Khan joined the project as a Research Associate (also began her MSc program) and will work on the *Ph1* gene and wheat transformation.

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NUCLEAR INSTITUTE OF AGRICULTURE (NIA)
Tando Jam, Pakistan.

Karim Dino Jamali.

Breeding for semidwarf habit and high grain yield in hexaploid wheat.

Wheat is an excellent crop for Pakistan and serves as staple food crop. To achieve high yield, raising productivity per unit area by adapting modern technologies such as high-yielding cultivars, improved cultural practices, and proper management of crop are needed. In 2006–07, an overall production of 23,520 x 10^6 t was achieved from an area of 8,493.9 x 10^6 ha (Table 1).

Table 1. Wheat area and production in Pakistan for the year 2006–07 (Source: Ministry of Food, Agriculture and Livestock, Islamabad Pakistan).

Province	Area (x 10^3 ha)	Production (x 10^3 tons)	Yield (kg/ha)
Punjab	6,393.1	17,850.0	2,792
Sindh	937.1	3,409.0	3,638
NWFP	754.8	1,390.0	1,842
Balochistan	408.9	871.0	2,130
Total	**8,493.9**	**23,520.0**	**2,769**

Wheat breeding at NIA, Tando Jam. Wheat breeding at the NIA is being pursued with the objectives of developing new cultivars endowed with high yield and good quality characters with tolerance to biotic and abiotic stresses. Currently, our breeding material is at different stages of evolution and is summarized below.

Performance of candidate cultivar 7-03. The candidate cultivar 7-03 has completed two years of National trials. The line tested in farmers' fields in performance trials. The candidate was tested in the Hyderabad, Matyari, Sanghar, and Khairpur districts. The line is resistant to both rusts according to CDRI (Cereal Disease Research Institute), Islamabad, in screening during 2006–07.