Genetic divergence in bread and durum wheat.

K.Thamayanthi and V.R.K. Reddy.

We evaluated 150 genotypes of bread and durum wheat, including Indian and exotic collections, for various agronomic characters following a nonhierarchical Euclidean cluster analysis. Genotypes were grouped into 13 clusters with a variable number of genotypes. Heterogenous genotypes of original place of release and different ploidy levels often grouped together in the same cluster, suggesting some degree of ancestral relationship between the genotypes. On the basis of the data on genetic divergence and mean performance of yield and other traits, five diverse and superior genotypes were selected, HI 1077, WH 147, WH 542, HD 2285, and UP 262. These genotypes may be involved in multiple crossing program to recover transgressive segregates.

HMW-glutenin subunit composition in Indian hexaploid wheat cultivars.

V.R.K. Reddy.

Thirty-two cultivars of bread wheat were analyzed for allelic variation in the HMW-glutenin subunits by SDS-PAGE. A total of nine alleles were identified in the 32 cultivars. At the *Glu-A1* locus, the alleles a and b encoded 1, 2-HMW-glutenin subunits. The HMW-glutenin subunits 2* was found in 22 of the 32 cultivars. Ten cultivars had subunit 1 (the a allele). At the *Glu-B1* locus, the alleles a, b, c, and d encoded glutenin subunits 7, 7+8, 7+9, and 6+8, respectively. Eight cultivars had glutenin subunit 7, four had subunit 7+8, 16 had the subunits 7+9, and four had subunits 6+8. At the *Glu-D1* locus, the alleles a, b, c, and d encoded HMW-glutenin subunits 2+12, 3+12, and 5+10, respectively. The *Glu-1* quality score 8 is present in a large number of cultivars.

CH. CHARAN SINGH UNIVERSITY
Molecular Biology Laboratory, Department of Genetics and Plant Breeding, Meerut-250 004, U.P., India.

P.K. Gupta, H.S. Balyan, J. Kumar, A. Mohan, A. Goyal, A. Kumar, R.R. Mir, S. Kumar, R. Kumar, S. Banerjee, and M. Das.

Development and use of molecular markers for wheat genomics and breeding.

Construction of framework linkage maps using trait specific intervarietal RIL populations. Three framework linkage maps using three mapping populations have been prepared in our laboratory for QTL interval mapping of various agronomically important traits. The three mapping populations were originally prepared for the following three traits: (i) grain protein content (GPC); (ii) grain weight (GW), and (iii) preharvest-sprouting tolerance (PHST).

Updating the framework linkage map of the GPC population. We earlier prepared a framework linkage map for the GPC population using 171 SSR markers. The map spanned a genetic distance of 3,272.4 cM and had large gaps in certain regions, which adversely affected the precision of QTL mapping studies. In view of this, the following two exercises were undertaken.

(a) Genotypic data on a set of 39 markers (including ISSR, SSR, and RAPD markers) was procured from the NCL, Pune (India), as a collaborative activity.

(b) An additional set of 124 SSRs was used to study polymorphism between parents of GPC population (WL711 and PH132). Forty-six of the above 124 SSRs showed polymorphism and were used for genotyping of RILs.

Using the genotypic data on above 85 markers, a total of 47 markers could be added to the existing framework map of GPC population. While updating the framework map, three markers from the existing map were eliminated, making the total number of markers in the map to 217. The map now spans a total genetic distance of 3,868 cM.

Framework map for the PHST population. A framework linkage map for a solitary chromosome (3A) was prepared earlier for the PHST population using genotyping data for 124 molecular markers (11 SSR, 76 AFLP, and 37 SAMPL) on 100 RILs of the above population. Only 13 of the 124 markers could be assigned to 3A, and an average genetic distance of 21.47 cM between any two markers was observed. This map of 3A was prepared for QTL interval mapping, because chromosome 3A is known to carry genes for PHST.

To develop the whole-genome framework linkage map of the PHST population, an additional 778 SSR primers were tested on parents of the mapping population, i.e., SPR8198 and HD2329. A total of 233 SSRs covering all the 21 chromosome of bread wheat were polymorphic between the two parents. These 233 SSRs were further used to screen a subset of 90 RILs of the mapping population. Further, 16 AFLP and 9 SAMPL primer combinations were tried for detection of polymorphism between the two parental genotypes; 23 AFLP and 91 SAMPL polymorphic markers were identified. The genetic map was composed of 214 loci (198 SSR, 5 AFLP, and 11 SAMPL loci), which were distributed on all 21 chromosomes with an average of 10.19 loci/chromosome. The map spanned a genetic distance of 3,972 cM. For all mapped loci, a maximum of 77 were in the A genome (11 loci/chromosome), followed by 73 loci in the B genome (10.42 loci per chromosome), and 64 in the D genome (9.14 loci/chromosome).

Framework map for the GW population. To prepare a framework linkage map for GW, a set of 836 primer pairs, including 337 WMC, 288 GWM, 90 BARC, 48 PK, 30 CFD, 25 CFA, and 18 GDM primers, were used to detect polymorphism between the two parent genotypes (RS111 and CS) of the RIL mapping population. Of the 836 SSR primer pairs, only 270 (32.3%) were found polymorphic between the two parental genotypes and were subsequently used for genotyping of the GW mapping population. In addition to SSR markers, 299 AFLP and 120 SAMPL polymorphic markers also were used. Using the genotyping data, a total of 294 loci, including 194 SSR, 86 AFLP, and 14 SAMPL loci, were mapped on all 21 chromosomes of wheat genome (average 14 loci/chromosome) covering a map length of 5,211 cM.

Genome-wide, single-locus and two-locus QTL analysis for PHST and GW. Using the data on the linkage map and PHS collected over six environments, genome-wide, single-locus and two-locus QTL analyses were conducted for preharvest sprouting tolerance. A single-locus analysis following composite interval mapping (CIM) revealed a total of seven QTL on 1A, 2A, 2D, 3A, and 3B, including one major QTL each on 2A and 3A. The PVE by individual QTL (R^2) ranged from 15.22% to 45.11%. Three of these QTL also were detected following two-locus analysis, which resolved a total of four main-effect QTL (M-QTL) and 12 epistatic QTL (E-QTL) involved in seven QTL × QTL interactions. These QTL could be efficiently utilized for marker-assisted selection for enhancing PHST in bread wheat.

For the genome-wide genetic dissection of GW in bread wheat, the genotypic data and GW data recorded on RILs over six environments (three locations × 2 years) were used for the genome-wide, single-locus QTL analysis using inclusive composite interval mapping (ICIM) and two-locus QTL analysis using QTL Network to identify main effect QTL (M-QTL) and epistatic QTL (E-QTL). Single-locus QTL analysis identified 11 QTL above threshold LOD values (3.95 to 32.0), which contributed significantly to the phenotypic variation (maximum PV in individual environments varied from 4.37% to 82.0%) for GW. These QTL included four major and stable QTL (explaining >20% PV; available in 50% environments), one each located on chromosomes 1A, 1B, 5A, and 6B. The major QTL on chromosome 1B (LOD value =10.7-32.0) explained a maximum (26.0-82.0%) PV in individual environments. Two-locus QTL analysis resolved a total of 30 QTL, which included three M-QTL (also detected by single-locus analysis) and 27 E-QTL involved in digenic Q × Q interactions; no Q × E and Q × Q × E interactions were detected. However, the level of PV explained by QTL identified through two-locus analysis was relatively low. The four, major QTL identified through single-locus analysis can be utilized for marker-assisted selection for improving GW in bread wheat.

Single- and two-locus QTL analysis for yield and yield contributing traits. The GPC and ITMI mapping populations were used to identify QTL for nine yield traits including plot yield and its components, using single- and two-locus QTL analysis. Framework linkage maps, consisting of 217 and 1,345 markers for the GPC and ITMI populations, respectively, and phenotypic data from four environments at two locations, were used for QTL analysis using QTL Cartographer and QTLNetwork software. Composite interval mapping using QTL Cartographer identified a total of 71 and 109 QTL located on 19 (except chromosome 5A and 6D) and 20 (except chromosome 7D) chromosomes in the GPC and ITMI populations respectively. QTLNetwork identified a total of 89 and 155 QTL, which included QTL with significant main effect and/or significant interaction effect (epistatic QTL or QTL involved in interaction with the environment), located

on 19 (except chromosome 6D and 7D) and 21 chromosomes in GPC and ITMI populations, respectively. In this study, a major QTL was identified on chromosome arm 2DS in both the GPC and ITMI populations for six yield traits each. In the ITMI population, this QTL was detected for plot yield, spike weight, spike length, spikelets/spike, seed weight, and 1,000-kernel weight (explaining from 13.00% to 37.85% PV for individual trait), whereas in the GPC population, this QTL was detected for plot yield, tiller number, spike length, spike compactness, number of seeds, and 1,000-kernel weight (explaining from 8.93% to 19.81% PV for individual trait).

The above QTL was physically mapped to distal bin (2DS5-0.47-1.00) covering 53% region of 2DS. Comparative mapping revealed that the genomic region with this QTL could be an orthologue of a major QTL for spikelets/panicle (*qSSP7*) located in a 912.4-kb region of rice chromosome 7. This information may prove useful for high-resolution mapping leading to map-based isolation of the above major QTL. The study revealed that epistatic interaction contributes significant portion of the phenotypic variation for these yield traits. Another important finding was that for about half (54 out of 106) of the epistatic interactions detected in both mapping populations, interactions between alleles from different parents (recombinant types) resulted in better trait values. This finding supports the suggestion that epistasis for yield traits in wheat may contribute to heterosis. In that case, marker-assisted selection can prove very successful to fix the portion of heterosis owing to QQ effects. The MAS strategy would, therefore, be a promising approach for utilizing heterosis. For some yield traits, environmental interactions also play an important role. The results of this study suggest that while selecting for increased yield, one also must pay attention to the best two locus QTL combinations as well as major genes and nonenvironment specific QTL.

Genetic diversity and population structure analysis among Indian bread wheat cultivars. As a first step towards association mapping in wheat, we analyzed the genetic diversity and structure in a collection of 134 Indian wheat cultivars that were released over a period of ~100 years (1910 to 2006). We used a set of 42 SSR markers, one each from each arm of 21 individual chromosomes. The 42 SSRs had a total of 257 alleles, which included 71 (27.6%) rare alleles occurring at a frequency of <5%. The number of alleles/locus ranged from 1 to 13, indicating considerable genetic diversity in the cultivars studied. The cultivars formed two groups, one with 31 cultivars released previous to the Green Revolution period and the other with 103 cultivars released after the Green Revolution period. The average number of alleles/locus in the cultivars from post-Green Revolution period was relatively higher (5.29 versus 4.76 alleles/locus), but genetic diversity did not differ (0.63, 0.62), indicating that Green Revolution did not lead to any loss of genetic diversity. Furthermore, analysis of molecular variance showed that the proportion of the variance among cultivars within groups accounted for 94.4% but between the groups only 5.6% of the overall molecular variance. The model-based, structure analysis identified a total of ten subpopulations including two subpopulations, from pre-Green Revolution cultivars and the remaining eight from post-Green Revolution cultivars.

An integrated physical map of 2,072 SSRs loci (gSSR and EST-SSRs). As many as ~2,800 genomic SSRs (gSSRs) and ~300 EST-SSRs have been genetically mapped so far world over. Of these, only 1,320 gSSRs have been physically mapped. As many as 270 of these mapped gSSRs and an additional set of 275 EST-SSRs (not used earlier for genetic/physical mapping) were physically mapped in our laboratory, which leaves a very large number of genetically mapped/unmapped gSSRs and EST-SSRs that are yet to be physically mapped. We extended our studies further, so that in our laboratory altogether we physically mapped as many as ~1,500 SSR loci (~800 gSSR loci + ~700 EST-SSR loci) involving all the 21 wheat chromosomes. This physical map was integrated with all other available SSR containing physical maps in wheat. In the integrated physical map, a maximum of 776 loci (37.45%) were mapped on B subgenome followed by D subgenome with 672 loci (32.43%) and A subgenome with 624 loci (30.11%).

To further enrich the physical map, we plan to map 132 class I gSSRs derived from the ~14 Mb of available genomic sequences belonging to wheat and its relatives (http://www.tigr.org//tdb/e2k1/tae1/info.shtm).

Molecular marker-assisted selection for improvement of GPC. Grain protein content is a major nutritional quality trait in bread wheat. Most of the Indian bread wheat cultivars have low to medium GPC (10.90% to 12.14%) and, thus, are relatively poor in their nutritional value. In order to improve the GPC of Indian bread wheat genotypes, a bread wheat genotype Yecora Rojo carrying a high GPC QTL (*GPC-B1*) was used as the donor parent in marker-assisted backcross breeding. For three successive backcross generations, the foreground selection for *GPC-B1* QTL was made using the STS marker *Xuhw89*, which is tightly linked (0.1 cM) to the *GPC-B1* QTL. Background selection was done using polymorphic markers developed by 35 SSRs (distributed on all the 21 wheat chromosomes) and AFLPs. In nine out of 10 BC_3F_1 populations, 2–5 positive plants with the *GPC-B1* QTL showing higher GPC (up to 1.72% higher than the recipient genotypes) and high genomic similarity (up to 100%) with the recipient parental genotype were selected.

In the remaining BC_3F_1 population, plants selected using MAS showed a decrease of 0.93% GPC over the recurrent parent genotype possibly as a result of interaction of high GPC QTL with the genetic background, which needs to be confirmed in future studies. Nevertheless, our results suggested improvement in GPC of the Indian bread wheat cultivars following introgression of *GPC-B1* QTL through MAS. Homozygous BC_3F_2 progenies for the above GPC QTL showing maximum genetic similarity with the recipient parent genotype will be isolated and evaluated in replicated field trials over environments for their agronomic performance.

Molecular marker-assisted selection for pyramiding of leaf rust resistance and PHST. Preharvest sprouting is a major problem word-wide that leads to degradation of grain quality associated with significant losses in yield. In view of this, we earlier identified a major QTL (*QPhs.ccsu-3A.1*) on chromosome 3A that explained >70% phenotypic variation for PHST across a number of environments. The desirable allele of this QTL was introgressed through MAS into the elite, but PHS susceptible, Indian bread wheat cultivar HD2329. HD2329 has the leaf rust-resistance genes *Lr24* + *Lr28*. In each of three backcrosses, foreground selection was performed using flanking markers (GWM155 and WMC153) and background selection was performed using polymorphic markers developed by a set of 35 SSRs (distributed on all the 21 bread wheat chromosome) and AFLPs. During introgression of PHST, the desirable alleles of two leaf rust-resistance genes *Lr24* and *Lr28* also were tracked using linked SCAR markers. In the BC_3F_1 generation, reconstituted plants were selected that exhibited 94.3-97.3% genetic similarity with the recipient bread wheat genotype and contained the QTL allele for PHST. Phenotypically, these plants exhibited a high level of PHS tolerance (PHS scores ranged from 1 to 3; 1= tolerant and 9= susceptible). These results validated the PHST QTL we identified earlier and suggested significant contribution of this QTL in conferring PHS tolerance. The selected plants will be advanced to BC_3F_2 in to obtain homozygous progenies for the PHST QTL. The homozygous and homogeneous progenies with high tolerance to PHS and with maximum genetic similarity with the recipient genotype will be evaluated in replicated field trials over environments.

Analysis of host–pathogen interaction in leaf rust infected bread wheat. Development of leaf rust-resistant cultivars is a major objective of wheat-breeding programs. For the long-term, effective management of resistance against this disease, the molecular basis of disease pathogenicity and the host–pathogen interaction should be known. In collaboration with BITS, Ranchi, and IARI, New Delhi, we have started a DBT-sponsored project in which the host–pathogen interaction in leaf rust-infected bread wheat will be analyzed using cDNA-AFLP display analysis. The cDNA-AFLP experiment will be conducted for the following two genes: (i) seedling-resistance gene *Lr28* (Thatcher NILs) and (ii) adult-plant resistance gene *Lr48* (Agra Local NILs). A single-spore derived 77-5 pathotype of *P. triticina* will be used for infection of wheat stocks. In cDNA-AFLP display analysis, differentially expressed transcripts will be identified and characterized. The above exercise of identification of differentially expressed transcripts will potentially lead to elucidation of specific signal transduction pathways that follow leaf rust-wheat interaction.

Acknowledgment. Financial support was provided by Department of Atomic Energy-Board of Research in Nuclear Science (DAE-BRNS), Department of Biotechnology (DBT), and Department of Science & technology (DST), Government of India.

Publications.

Balyan HS, Sreenivasulu N, Riera-Lizarazu O, Azhaguvel P, and Kianian SF. 2008. Mutagenesis and high-throughput functional genomics in cereal crops: current status. Adv Agron 98 (In press).

Gupta PK. 2006. RNA interference – gene silencing by double-stranded RNA: The 2006 Nobel Prize in Physiology or Medicine. Curr Sci 91:1443-1446.

Gupta PK. 2006. Plant cytogenetics: A re-birth in twenty-first century. Ind J Crop Sci 1:1-7.

Gupta PK. 2006. Pyramiding of genes/QTLs for crop improvement using marker-assisted selection (MAS). *In:* Proc Dr. B.P. Pal Birth Centenary Symp, NAAS, New Delhi, India. Pp. 333-364.

Gupta PK. 2006. New frontiers in cytogenetics research (based on Birbal Sahni Medal Award Lecture). J Ind Bot Soc 85:1-11.

Gupta PK. 2007. Quantitative genetics on the rise. Curr Sci 93(8):1051-1052.

Gupta PK. 2007. Epigenetics: An overview. Proc Natl Acad Sci India (special issue) 77(B):1-7.

Gupta PK. 2007. Transgenerational inheritance of epigenetic variation. Proc Natl Acad Sci India (special issue) 77(B):9-18.

Gupta PK. 2007. RNAi-mediated gene silencing and epigenetics. Proc Natl Acad Sci India (special issue) 77(B):51-60.

Gupta PK. 2007. Ultrafast and low-cost DNA sequencing methods for applied genomics research. Proc Natl Acad Sci India (In press).
Gupta PK. 2007. Pyramiding genes/QTL for crop improvement using marker-aided selection (MAS). *In:* Search for New Genes (Chopra VL, Sharma RP, Bhat SR, and Prasanna BM, Eds). Academic Foundation, New Delhi, India. Pp. 145-171.
Gupta PK, Balyan HS, Kulwal PL, Kumar N, Kumar A, Mir RR, Mohan A, and Kumar J. 2007. QTL analysis for some quantitative traits in bread wheat. J Zhejiang Univ SCIENCE B 8(11):807-814.
Gupta PK, and Kulwal PL. 2006. Methods of QTL analysis in crop plants: present status and future prospects. *In:* Biotechnology and Biology of Plants (Trivedi PC, Ed). Avishkar Publishers, Jaipur, India. Pp. 1-23.
Gupta PK, Mir RR, Mohan A, and Kumar J. 2008. Wheat Genomics: present status and future prospects. Internat J Plant Genomics. Special issue on genomics of Major Crops and Model Plant Species, Hindawi Pub Corp, USA (In press).
Gupta PK, Rustgi S, and Kumar N. 2006. Genetic and molecular basis of grain size and grain number and its relevance to grain productivity in higher plants. Genome 49:565-571.
Gupta PK, Rustgi S, and Mir RR. 2008. Array-based high-throughput DNA markers for crop improvement. Heredity (In press).
Kumar J and Gupta PK. 2008. Molecular approaches for improvement of medicinal and aromatic plants. Plant Biotech Rep (Accepted).
Kumar J and Gupta PK. 2008. Non-transgenic molecular approaches for improvement of *Cymbopogon* species for aromatic and medicinal uses: current status and future prospects. *In:* Medicinal Plants: Research Progress & Environmental Concerns (Iqbal M, Ed) (Accepted).
Kumar N, Kulwal PL, Balyan HS, and Gupta PK. 2007. QTL mapping for yield and yield contributing traits in two mapping populations of bread wheat. Mol Breed 19:163–177.
Kumar N, Kulwal PL, Gaur A, Tyagi AK, Khurana JP, Khurana P, Balyan HS, and Gupta PK. 2006. QTL analysis for grain weight in common wheat. Euphytica 151:135-144.
Kumar J, Verma V, Qazi GN, and Gupta PK. 2007. Genetic diversity in *Cymbopogon* species using PCR-based functional markers. J Plant Biochem Biotech 16:119-122.
Kumar J, Verma V, Qazi GN, and Gupta PK. 2007. Genetic Diversity in *Cajanus-Rhyncosia*- Flemingia group based on functional markers. Proc Natl Acad Sci India 77:269-274.
Mir RR, Rustgi S, Sharma S, Singh R, Goyal A, Kumar J, Gaur A, Tyagi AK, Khan H, Sinha MK, Balyan HS, and Gupta PK. 2008. A preliminary genetic analysis of fibre traits and the use of new genomic SSRs for genetic diversity in jute. Euphytica DOI:10.1007/s10681-007-9597-x.
Mohan A, Goyal A, Singh R, Balyan HS, and Gupta PK. 2007. Physical mapping of wheat and rye EST-SSRs on wheat chromosomes. The Plant Genome, a supplement to Crop Sci 47:S-1-S13.

DIRECTORATE OF WHEAT RESEARCH
Regional Station, Flowerdale, Shimla-171002, India.

Genetic basis of stripe rust seedling resistance of Cappelle-Desprez and Mega.

D. Datta, M. Prashar, and S.C. Bhardwaj.

Introduction. Wheat is grown under diverse environments and different agroecological systems. Apart from the inherent yield potential both biotic and abiotic stresses also determine the realized yield of cultivars. Stripe rust or yellow rust of wheat is an important cereal rust disease in many wheat-growing regions of the world, especially in areas with cool and wet environmental conditions (Roelfs et al. 1992). Rust diseases can be managed effectively and economically in a eco-friendly manner through cultivation of resistant cultivars (Line and Chen 1995). Understanding the genetic basis of resistance is of prime importance for their use in breeding program and not only generates information about the nature and number of genes in the donor parents but also helps in formulating efficient strategy for the incorporation of rust resistance. The present investigation was initiated with the objectives of understanding the genetic basis of stripe rust resistance of some the very important winter wheat cultivars. The results of genetic analysis of stripe rust resistance of Cappelle-Desprez and Mega is discussed.