genotypes also were subjected to an SSR analysis to find molecular similarities among the genotypes. Further analysis is underway to deduce the genetic relationships and commonalities based on quantitative, parentage, and SSR data.

Wheat tissue culture.

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Calli were induced from scutellum-supported embryos of immature seeds in three lines of *T. turgidum* subsp. *dicoccum*, two cultivars of *T. aestivum* subsp. *aestivum*, and two experimental stocks with the sphaerococcum trait. Differences in growth rates of the calli from different cultivars were observed. Calli from experimental stocks carrying the sphaerococcum trait were smaller than the rest. Calli obtained from the scutellum-supported embryos of mature seeds in four cultivars of *T. aestivum* subsp. *aestivum* and the two experimental stocks showed that the growth rate of the calli from experimental stocks carrying sphaerococcum trait were significantly lower.

Calli obtained from scutellum-supported embryos of immature seeds were irradiated with gamma rays. Three days after irradiation, the calli were assayed using TTC (2,3,5-triphenyl tertazolium chloride). At 50 Gy, the reduction in TTC values for Unnath C306 (*T. aestivum* subsp. *aestivum*) was 9% and for DDK1029 (*T. turgidum* subsp. *dicoccum*) was 1%. A 65% and 68% decrease in the TTC value of Unnath C306 and DDK1029, respectively, were observed after a 500-Gy treatment.

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Development and use of molecular markers for wheat genomics and breeding

Construction of framework linkage map(s) 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. These three mapping populations originally were prepared for the following three traits: (i) grain protein content (GPC); (ii) preharvest-sprouting tolerance (PHST), and (iii) grain weight (GW).

Framework linkage maps of GPC, PHST, and GW populations. Previously, we 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, a total of 47 markers were added to the existing framework map of the GPC population making the total number of markers in the map 217. The map now spans a total genetic distance of 3,868 cM.

For the PHST population, the genetic map that was prepared consisted of 214 loci (198 SSR, 5 AFLP, and 11 SAMPL loci) that were distributed on all 21 wheat chromosomes with an average of 10.19 loci/chromosome. The map spanned a genetic distance of 3,972 cM. Of the total mapped loci, a maximum of 77 loci were mapped to the A genome (11 loci/chromosome), followed by 73 loci to the B genome (10.42 loci per chromosome), and 64 loci to the D genome (9.14 loci/chromosome).

For the GW population, a total of 294 loci, including 194 SSR, 86 AFLP, and 14 SAMPL loci, were mapped on all the 21 chromosomes of wheat genome (average 14 loci/chromosome) covering a map length of 5,211 cM. SSRs were more abundant in the A genome (110 SSR loci with an average of 15.7 loci per chromosome) than either the B (103 SSR loci with an average of 14.7 SSR per chromosome) or D genomes (81 SSRs with an average of 11.57 SSR per chromosome).

QTL analysis for grain weight and related kernel traits in bread wheat. Kernel size and shape are important traits in bread wheat because of their relationship with yield and milling quality. For the genome-wide genetic dissection of some kernel traits in bread wheat (kernel size including 1,000-kernel weight (GW) and kernel shape), an intervarietal RIL mapping population derived from the cross 'Rye Selection 111/Chinese Spring' was used. Kernels from Rye Selection 111 are larger than kernels from Chinese Spring in all dimensions. The two parental genotypes and RILs were evaluated in six environments for GW; for other traits (kernel length, width, volume, projection area, vertical perimeter, and horizontal axis proportion), the data was recorded over three environments. Digital image analysis was used for recording the data. Using genotypic and phenotypic data, genome-wide, single-locus QTL analysis (involving inclusive composite interval mapping (ICIM)) and two-locus QTL analysis (involving QTLNetwork) were used to identify the main effect QTL (M-QTL), epistatic QTL (E-QTL), and 'QTL × environment' interactions (QE and QQE). Single-locus QTL analysis for GW revealed a total of 11 QTL (including four major and stable QTL). Threshold LOD scores (3.95 to 32.0) were used to score QTL that contributed significantly to the phenotypic variation (PV = 4.37% to 82.0% per QTL). Similarly, for other related traits, a total of 45 QTL were identified (ranging from four for the vertical perimeter to 13 for kernel length) above threshold LOD values (2.52 to 9.27), which contributed significantly to phenotypic variation (PV = 6.97 for kernel length to 29.87 for projection area). Among the above QTL for GW and related traits, 11 were found to control more than one trait (including four for GW) and were, therefore, considered as pleiotropic/coincident QTL. A two-locus QTL analysis for GW resolved a total of 24 QTL, which included three M-QTL (also detected by single-locus analysis) and 21 E-QTL involved in 12 digenic QQ interactions. Similarly for other traits, a total of 35 QTL including seven M-QTL (five of the seven also were detected through ICIM) and 28 E-QTL involved in 15 digenic QQ and two QQE interactions were detected. The molecular markers linked with the major/coincident QTL for GW and other traits may prove useful in marker-assisted selection for the development of improved bread wheat cultivars.

QTL analyses for grain color and preharvest sprouting. Using the GPC population, single- and two-locus QTL analyses resolved a total of 11 QTL for PHS and 12 QTL for GC. These QTL included both the main-effect QTL (M-QTL; seven for PHS and six for GC) and epistatic QTL (E-QTL; four for PHS and six for GC). The MQTL explained a greater proportion of phenotypic variation (PV) than the E-QTL for both the traits. Four QTL for each of the two traits were co-localized, whereas the remaining M-QTL and E-QTL were unique for each of the two traits. Of all the QTL, one major QTL each for PHS and GC are of interest for breeding PHS-tolerant, white-grained, bread wheat genotypes. The major QTL for PHS, which is independent of grain color, was located on chromosome arm 6AL and explained up to 29.47% PV, whereas the major QTL for GC, co-localized with a minor QTL for PHS, was located on chromosome arm 3BL and explained up to 36.18% PV. Physical mapping placed the QTL for PHS within the 53% proximal region of 6AL, whereas the QTL for GC was placed within 19% of the distal region of 3BL. Comparative genomic analysis also identified 5.47 Mb and 1.63 Mb rice genomic regions, which are orthologous to the wheat genomic regions containing the major QTL for PHS and GC, respectively. SSR markers flanking the major QTL for PHS and GC may be used in wheat-breeding programs aimed at developing PHS-tolerant, white-grained wheat genotypes through MAS. Further-

more, the information gained from physical and comparative mapping may be used in the future for fine mapping and map-based cloning of the above two major QTL.

Genetic diversity and population structure analysis among Indian bread wheat cultivars. As a first step towards association mapping in wheat, we carried out genetic diversity and structure analyses in a collection of 263 Indian wheat cultivars (45 developed during before the Green Revolution and 218 developed during thew post-Green Revolution period) that were released over a period of ~100 years (1910 to 2006). For this purpose, we used a set of 42 SSR markers, one from each arm of the 21 individual chromosomes. The above 42 SSRs had a total of 294 alleles (mean 7.0; range 2–14/SSRs), which included 101 (34.35 %) rare alleles occurring at a frequency of <5%. The average number of alleles/locus (5.91 vs. 5.74) and the estimates of genetic diversity (0.65 vs. 0.61) in the cultivars belonging to pre-and post-Green Revolution periods did not differ significantly indicating that the Green Revolution did not lead to any loss of genetic diversity. The model-based *Structure* analysis identified a total of 14 subpopulations including two subpopulations predominantly comprising cultivars from the pre-Green Revolution period and 12 subpopulations mostly comprising cultivars from post-Green Revolution period.

Introgression of QTL for GPC using MAS. Ten F_1 hybrids were derived from the crosses of each of the 10 elite, Indian, bread wheat genotypes with a high GPC donor genotypes and Yecora Rojo, carrying a major QTL for GPC (*GPC-B1*). The F_1 hybrids were backcrossed with the respective elite recipient parental genotype and the BC_1F_1 plants were raised either in off-season nursery at National Phytotron Facility, IARI, New Delhi during 2004–05 or in the rabi season 2005–06 at the Research Farm of CCS University, Meerut. From the BC_1F_1 onwards, MAS (foreground and background selection) was exercised for three successive backcross generations for the rapid introgression of high GPC QTL and reconstruction of the recipient genotypes. The foreground selection for *GPC-B1* QTL was carried out using STS marker *Xuhw89*, which is tightly linked (0.1 cM) to the *GPC-B1* QTL. Background selection for the recovery of the recurrent parent genotype was carried out using 35 SSRs (representing 52 polymorphic loci) and 12 AFLP primer combinations (889 polymorphic AFLP loci). In each of the 10 BC_3F_1 populations, 2–5 positive plants carrying *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 BC_3F_2 generation, progenies of the six crosses could be advanced in National Phytotron Facility at IARI during the off-season 2006–07, and the BC_3F_3 seed from 29 plants homozygous for the GPC-B1 QTL (identified following foreground selection) was obtained. Using BC_3F_3 seed, 29 BC_3F_3 progenies (belonging to six crosses) were evaluated in five replications at the Research Farm of CCS University, Meerut, during the rabi season 2007–08. Out of these progenies, 17 progenies showed significantly higher GPC (1.08 to 2.51%) over their respective recipient parent genotypes. These progenies with significantly higher GPC did not show any adverse effect of increased GPC on tiller number, spike traits, and 1,000-kernel weight, although some of these progenies did show reduction in plant height. The 17 progenies (BC_3F_4) with significantly higher GPC were evaluated in 2-m single-row plots in five replications at each of the three different locations (Meerut, Pantnagar, and Ludhiana) during the rabi season 2008–09 and the data on six agronomic traits (plant height, spike length, number of spikelets/spike, number of seeds/spike, seed weight/spike, and 1,000-kernel weight) were recorded. Out the above 17 BC_3F_4 progenies, 10 BC_3F_4 progenies were evaluated in separate yield trail in 2-m^2 plots in three replications at Meerut during 2008–09. The data were recorded on grain yield and six agronomic traits. Efforts are underway to record the data on GPC of all the progenies in both the trials. Superior BC_3F_4 progenies will be identified after complete data on all the traits is obtained. In addition to the above, BC_3F_2 progenies of the nine crosses (including four crosses involving the recipient genotypes that were not involved in the above BC_3F_4 progenies) were evaluated at the Research Farm of CCS University, Meerut, during the rabi-season 2007–08. Following foreground selection, 99 plants homozygous for the *GPC-B1* QTL were selected and their progenies (BC_3F_3) were evaluated in 2-m, single row plots in five replications during the rabi season 2008–09 at the Research Farm of CCS University, Meerut. Data were recorded on six agronomic traits (plant height, spike length, number of spikelets/spike, number of seeds/spike, seed weight/spike, and 1,000-kernel weight) on these progenies. Data on GPC will be recorded soon. The BC_3F_3 progenies showing significantly higher GPC and high genomic similarity with the recipient parent genotypes will be evaluated in the future in replicated/multilocation trials for GPC and yield-related traits.

Marker-assisted selection for preharvest sprouting tolerance and leaf rust resistance in bread wheat. In wheat, preharvest sprouting and susceptibility to leaf rust are two major problems that lead to the degradation of grain quality associated with significant losses in yield. 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, amber-grained wheat cultivar HD2329 carrying alien leaf rust-resistance genes (*Lr24 + Lr28*). In each of the backcross generation, foreground selection was exercised using

flanking markers (gwm155 and wmc153), and background selection was performed using 52 polymorphic SSR loci (distributed on all the 21 bread wheat chromosomes) and 146 AFLP loci. In the BC_3F_1, the desirable alleles of the two leaf rust-resistance genes *Lr24* and *Lr28* also were tracked using linked SCAR markers. The reconstituted plants, exhibiting upto 93.4 3% genetic similarity with the recipient parent, were selfed to obtain homozygous plants in the BC_3F_2, which were further evaluated in the BC_3F_3. Seven lines with pyramided PHST QTL and Lr genes exhibited high level of PHS tolerance (PHS score 2–4) and resistance against leaf rust under artificial conditions. The present work demonstrates successful application of marker-assisted selection for targeted pyramiding of QTL/genes for more than one trait into an improved wheat cultivar.

Introgression of QTL for GW using MAS. Crosses involving 10 elite Indian bread wheat genotypes as recipient parents and the genotype Rye Selection 111 (RS111) as a donor parent were attempted during the off-season 2005–06 in the Phytotron Facility at IARI, New Delhi ,and the F_1 seed was collected. These F_1s were raised during the rabi season 2006–07 and were backcrossed with their respective recurrent parents to obtain the BC_1F_1 seed. A total of 470 BC_1F_1 seeds belonging to five crosses [RS111/HD2329, PBW343 (*Lr9*)/RS111, HI977/RS111, K9107/RS111, and RAJ3765/RS111] were obtained. Using above seed material, ~259 BC_1F_1 plants were raised during rabi 2007–08. Following foreground selection, 27 positive plants for markers *Xwmc24* and *Xwmc59* (associated with two separate QTL for GW on chromosome 1A), 127 positive plants for the marker *Xwmc24*, and 57 positive plants for the marker *Xwmc59* were selected. The selected BC_1F_1 plants were backcrossed with their respective recurrent parents and BC_2F_1 seeds was obtained, which were used to raise BC_2F_1 progenies in the field during the rabi season 2008–09. Following foreground selection, three positive plants for both the markers *Xwmc24* and *Xwmc59* (associated with two separate QTL for GW on chromosome 1A) involving recipient genotype PBW343 (*Lr9*), 142 positive plants for the marker *Xwmc24* involving recipient genotypes PBW343 (*Lr9*), K9107 and Raj3765, and 18 positive plants for the marker *Xwmc59* involving recipient genotype PBW343 (*Lr9*) were selected. The selected plants were backcrossed with their respective recurrent parents to obtain BC_3F_1 seed, which will be used to raise BC_3F_1 progenies during 2009–10 rabi season.

Orthology between genomes of *Brachypodium*, wheat, and rice. Comparative sequence analysis of 3,818 *Brachypodium* EST (bEST) contigs and 3,792 physically mapped wheat EST (wEST) contigs revealed that as many as 449 bEST contigs were orthologous to 1,154 wEST loci that were bin-mapped on all the 21 wheat chromosomes. Similarly, 743 bEST contigs were orthologous to specific rice-genome sequences distributed on all the 12 rice chromosomes. As many as 183 bEST contigs were orthologous to both wheat and rice genome sequences, which harbored as many as 17 SSRs conserved across the three species. Primers developed for 12 of these 17 conserved SSRs were used for a wet-lab experiment, which resolved relatively high level of conservation among the genomes of *Brachypodium*, wheat, and rice. The study thus confirmed that *Brachypodium* is a better model than rice for analysis of the genomes of temperate cereals like wheat and barley. The whole-genome sequence of *Brachypodium*, which should become available in the near future, will further facilitate greatly the studies involving comparative genomics of cereals.

Analysis of host–pathogen interaction in leaf rust-infected bread wheat. One major objective in wheat-breeding programs is the development of leaf rust-resistant cultivars. However, for the long-term, effective management of resistance against this disease, the molecular basis of disease resistance and the host-pathogen interaction should be known. For the above purposes, we have attempted both *in silico* and wet-lab approaches to study transcriptome analysis.

***In silico study*.** Transcript based UniGene sets provide great potential to identifying the differentially expressed genes upon infection with leaf rust in bread wheat. Three, wheat cDNA libraries containing ~51,000 ESTs were utilized in the present study. The first cDNA library of the uninfected, disease-resistant Thatcher wheat stock (background gene *Lr10*) contained 22,803 ESTs. The second cDNA library of an infected, disease-resistant Thatcher wheat (background gene *Lr10*) contained 22,740 ESTs, and the third cDNA library of an infected, disease-resistant Thatcher wheat stock (background gene *Lr1*) contained 6,698 ESTs. Using the ESTs belonging to the three libraries, digital gene-expression analysis was conducted with the help of Digital Differential Display (DDD) program available at NCBI. Using this approach, a total of 68 differentially expressed UniGenes were identified, which formed three major clusters, each cluster representing a different class of genes including biotic and abiotic stress responsive genes as well as regulatory genes.

Wet-lab study. For transcriptome analysis of seedling resistance provided by the gene *Lr28*, total RNA was isolated from seven-day-old seedlings of each of the resistant (HD2329 + *Lr28*) and susceptible (HD2329) wheat stocks (a) before inoculation, i.e., at 0 h; (b) at 48 h, 96 h, and 168 h after inoculation with leaf rust pathogen race 77-5; and (c) at 168 h after the mock inoculation. Using the above RNA samples, high-quality cDNA samples were obtained. These cDNA samples were utilized to study the transcript derived fragments (TDFs) following cDNA–AFLP analysis using 17

*Eco*RI+3/*Mse*I+3 γP^{32} labeled primer combinations. Following cDNA-AFLP analysis, over-expressed TDFs in the resistant host following pathogen inoculation are being cloned and sequenced for their functional analysis.

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Preservation of wheat and barley germ plasm under natural storage conditions.

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We preserved seeds of wheat and barley germ plasm for long periods under the natural conditions of Dalang Maidan in the Lahaul Valley to reduce the exorbitant costs of installation and maintenance of artificial and conditioned storage rooms for germ plasm preservation. We also aimed to develop a standby repository for valuable Indian wheat and barley germ plasm presently being maintained in artificial storage rooms at the NBPGR, New Delhi, and DWR, Karnal. Accidental lapses leading to complete seed lethalities in these storerooms always loom large owing to unexpected human error, natural calamities, or electrical failure.

For every 1% decrease in seed moisture and 10°F in storage temperature, the life of seed is doubled (Harrington and Douglas 1970). Therefore, an attempt was made to store wheat and barley germ plasm under natural conditions characterized with low humidity and low temperature year round, at the Regional Station, Directorate of Wheat Research (ICAR), Dalang Maidan, in district Lahaul Spiti (Himachakl Pradesh). This station is located at approximately 32°.21' north latitude and 77°.14' longitude and is about 6-km upstream from the point of origin of the Chenab River, on the banks of the Chandra River at an altitude of approximately 3,300 M (10,000 ft) above mean sea level. This place is located at a very high altitude and enjoys a temperate-dry climate with an average annual rainfall of 250 mm. The area is covered with snow for about 5 months; from December to April.

Temperatures above 20°C during the summer months are a rare phenomenon, even at lower elevations. Records of the natural temperature and relative humidity (monthly means) pertaining to the room used to store the experimental seeds were maintained. The mean monthly temperature inside the storage room varied within a range of –20–20°C, and humidity levels were not above 60% during the 10-year span of the experiment (Table 1, p. 72).

Routinely during the last decade, new germ plasm accessions developed under national wheat program (All India Coordinated Wheat & Barley Improvement Programs) and those collected from international sources are packed in alkathane pouches, arranged together in plastic trays, and stacked in steel boxes at room temperature. This experiment