ITEMS FROM INDIA

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Current activities: Genetic improvement for rust resistance and quality traits in Indian wheat.

B.K. Das and S.G. Bhagwat.

Rust resistance genes, such as *Sr31*/*Lr26*/*Yr9*, *Sr26*, *Sr24*/*Lr24*, and *Lr34*, and specific HMW-glutenin subunits are being recombined with good agronomic traits. Selected lines from several intervarietal crosses are in different generations (F_2–F_5) and are being evaluated. Marker-assisted selection is being used to screen for specific rust resistance genes.

Using induced mutations, some early flowering mutants in the cultivars C-306 and MP-3054 were isolated and are being further evaluated. The early mutants were crossed with HW-2004 (C-306 + *Sr24*/*Lr24*) to recombine earliness and rust resistance. With the aim of isolating mutants resistant to rust diseases, the cultivars PBW343 and NI917 were mutagenized with gamma rays and populations from the M_1 generation were grown.

Marker-assisted backcrossing is being used to improve the rust resistance and dough strength of HD2189 wheat by incorporating the *Lr24*/*Lr24* and *Glu-D1d* genes. Eighteen BC_4F_1 plants were grown, and DNA from leaves of four-week old individual plants was extracted and screened using SCAR markers for these two genes. In the winter of 2009–10, five plants carrying both markers were identified. Backcrosses were made using the recurrent parent HD2189 and carriers of both the markers.

Marker-assisted selection to combine rust resistance genes (*Sr24* and *Sr26*) and *Glu-D1d* (coding for HMW-glutenin subunits 5+10) is being carried out in a cross between FLW-2 and Kite. In the F_2 generation, ~220 plants were analyzed using SCAR markers. Plants carrying markers for rust resistance genes *Sr24* and *Sr26* and *Glu-D1d* were selected and will be evaluated for their field performance.

Validation of a SCAR marker (Sr26#43) for stem rust resistance gene Sr26 in Indian wheat genotypes and segregating populations.

B.K. Das, Ruchi Rai, and S.G. Bhagwat.

Stem rust is a potential threat to the wheat crop and causes significant losses worldwide. Ug99, a race of black stem rust detected in Uganda, shows virulence to a great majority of wheat cultivars. The stem rust resistance gene *Sr26* is translocated to wheat from *Thinopyrum elongatum* and no virulence towards the *Sr26* gene has been reported. A SCAR marker, Sr26#43, was reported for this gene by Mago et al. (2005). To validate this marker in Indian wheat genotypes, 49 wheat genotypes were screened using SCAR marker Sr26#43. Analysis of these genotypes showed that the SCAR marker was present in all the genotypes carrying *Sr26*, except HW2090, which was reported to carry *Sr26* gene. The marker was absent in the genotype that lacked *Sr26* or carried any other stem rust resistance genes.

Two F_2 populations from crosses involving susceptible (Kalyansona (-*Sr26*) and resistant (Kite (+*Sr26*) and Takari (+*Sr26*) genotypes were used for validation. The phenotypic rust reaction data and marker data matched one-to-one, indicating that this marker can be used in early generations to selecte for the *Sr26* gene. Incorporating this gene is recommended to prevent stem rust epidemics caused by Ug99. The validated marker Sr26#43 will facilitate incorporating this gene in new breeding lines. The durability of *Sr26* can be enhanced by pyramiding it with other rust resistance genes. Multiplex PCR for the simultaneous screening of *Sr26* and *Sr24* is in progress.

The help of the DWR Regional Station, Flowerdale, Shimla, for phenotypic screening of some F_3 lines is acknowledged. The genotypes carrying *Sr26* were provided by DWRRS, Shimla, and IARIRS, Wellington. During this period, Shri. K. Arun participated in some of the experiments as project trainee.

Analysis of semidwarfing genes and polymorphisms at the Xgwm261 locus in a recombinant inbred population of bread wheat.

Suman Bakshi and S.G. Bhagwat.

Recombinant inbred lines (RILs) derived from a cross between cultivars Sonalika and Kalyansona in the F_9 generation were grown in the winter season of 2009–10. Leaves of one individual from each line were harvested and used for DNA extraction. The parental cultivars and the RILs were analyzed for the presence of *RhtB1b* and *RhtD1b* using perfect markers (Ellis et al. 2002). Variation at the microsatellite locus *Xgwm261* was studied. The parent cultivar Kalyansona had a 192-bp allele; the other parent Sonalika had a 165-bp allele. The RILs showed a 1:1 ratio for the presence of these alleles. Culm height was recorded on the RILs by measuring the culm of the main tiller of five plants when the plants were near maturity. The results showed that the RILs carrying *Rhtb1a* and *RhtD1a* were the tallest, followed by those with *RhtB1b* and *RhtD1b*. Plants with both semidwarfing genes were shortest. The RILs with a given a *Rht* gene composition were further classified according to the presence of *Xgwm261*. The results indicate that there was no reduction in culm height associated with the presence of the 192-bp allele. Further analysis is in progress.

Canopy temperature depression studies in bread wheat.

Heat stress is one of the most important stresses in subtropical, wheat-growing areas of the world and results in grain yield losses. The stage at which the wheat crop faces heat stress varies with the location and cropping season. In some areas, the stress is experienced at either at the seedling stage or at the grain-filling stage, in other cases the stress is felt through out the life of the plant. Heat stress affects the crop by altering many traits. Wheat cultivars differ in their canopy architecture, and this may result in differences in canopy temperature. Canopy temperature depression, the difference between air temperature and canopy temperature, can be measured. An experiment was carried out at the experimental field in Trombay in the winter of 2009–10. Seventeen wheat cultivars, which included both heat stress tolerant and susceptible cultivars, were grown in a replicated experiment. Canopy temperature was measured with an infrared thermometer. Measurements were made around 12:00 PM from tillering to flag leaf senescence at weekly intervals. At harvest, data on agronomic parameters were recorded using five plants from each replicate of each cultivar. Canopy temperature values appeared to vary across cultivars and growth stages. Data are being analyzed.

Threshability in recombinant inbred lines of bread wheat.

S.G. Bhagwat.

In wheat, threshability is an important trait. Very soft glumes and loose attachment to the rachis results in deciduous glumes that fall off if the spikes are not harvested in a timely fashion resulting in some grain loss. Thick glumes, with a strong attachment to rachis, make threshing hard. Tough glumes are associated with a brittle rachis, which is know as the nonfreethreshing habit.

Studies on the genetics of tough glumes and brittle rachis have been reported. Using interspecific crosses, QTL for threshability have been identified. Crosses between semi-wild and common wheat indicated that the fragile rachis and nonfreethreshing character of semi-wild wheat are dominant to the tough rachis and freethreshing character of common wheat. Rachis fragility and glume tenacity of semi-wild wheat were each controlled by a single gene (Cao et al. 1997). In hexaploid wheat, the glume tenacity gene *Tg* and *Q* locus control threshability. The *Tg* gene was mapped on 2DS of *T. aestivum* in the distal region (Sood et al. 2009). RILs evaluated for kernel shattering, glume strength, glume-pair angle, open-floret percentage, spike density, and plant height in different environments showed that glume strength consistently correlated with kernel shattering in all test environments, but their correlation was moderate. One QTL for glume strength was identified in the genomic regions containing the kernel-shattering QTL, suggesting that glume

strength is not the only genetic factor that determines kernel shattering. These results indicate that glume pair angle and open floret percentage might be the direct causes of kernel shattering (Zhang et al. 2009).

We are developing RILs from a cross between the cultivars Sonalika and Kalyansona, and RILs in the F_9 generation were grown in field. Single spikes were harvested at maturity, threshed by hand, and classified according to their ease or difficulty in threshing. Kalyansona was easier to thresh than Sonalika. The RILs varied for the trait. Lines easier to thresh than Kalyansona and harder to thresh Sonalika were observed. Each line was given a major category rating as follows: 1, deciduous glumes or very soft threshing; 2, similar to cultivar Kalyansona; 3, similar to cultivar Sonalika; 4, tougher glumes and hard to thresh; and 5, tough glumes very hard to thresh. Data were taken on 138 RILs in 2009–10. Based on the hand feel, the RILs were given scores in between the major categories mentioned above (Table 1).

Table 1. Scoring of glume and threshing traits in field-grown, F_9 RILs between the cultivars Sonalike (tough threshing) and Kalyansona (freethreshing).

Description	Rating	Frequency
Very soft and deciduous	1.0	00
Intermediate	1.5	09
Kalyansona type	2.0	31
Intermediate	2.5	12
Sonalika type	3.0	40
Intermediate	3.5	22
Tougher glumes, hard threshing	4.0	14
Intermediate	4.5	05
Tough glumes, very hard threshing	5.0	05

The data showed transgressive segregation for the trait. Observations also were taken in the F_7 and F_8 generations in 2007–08 and 2008–09, respectively, however the RILs were not rated as in 2009–10. Of the 18 RILs that were rated 4.0 or above in 2009–10, 14 were rated as hard or medium hard to thresh in 2007–08 and 12 were rated as hard or medium hard to thresh in 2008–09. Five lines were rated as soft in 2007–08 and four in 2008–09. Of the 40 lines that were rated from 1.0 to 2.0 in 2009–10, data on 26 were available from 2008–09; 24 were rated soft and two were rated as hard or medium hard. In 2007–08, 37 were rated as soft threshing, and three were rated hard or medium hard. These results indicate that some consistency between years. The disagreement could be due to error in judgment, environmental variation, or segregation.

Rachis breaking on 138 RILs was recorded in 2009–10. Fragile rachis was observed in 19 lines, the rachis remained intact in 98 cases, and was intermediate in 21. Of the 19 lines rated as fragile, 13 rate 4.0–5.0, indicating that the fragile rachis was largely accompanied by tougher glumes and hard threshing. Two lines with fragile rachis were rated 1.0, 3.0, and 3.5.

The RILs and parents were classified according to number of spikelets/cm of spike length. This value indicated whether the spike was compact or lax. Kalyansona showed a more compact spike with 2.47spikelets/cm; Sonalika had 1.72 spikelets/cm. RILs with lax spikes were more frequent than those with denser spikes. Fifty-six percent of the RILs were in the category of less than or equal to the Sonalika parent. More compact spikes (with 2.0 or more spikelets/cm) were observed in 30% of the RILs.

The RILs with denser spikes were classified according to their threshability rating. Of the 43 RILs, 18 were easy to thresh (rating 2.0 or lower), 21 were in the medium range (rating 2.5 to 3.5), and four were in the hard threshing range (rating 4.0 or more). The rachis remained intact in 31 of the 43 RILs, was intermediate in seven, and fragile in five. These results showed that there was an incomplete association between high spike density and easy threshability or nonfragile rachis. These RILs originated from intervarietal crosses and could be useful in identifying loci governing threshability trait in bread wheat.

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Deployment of molecular markers for the improvement of some important quality traits in bread wheat.

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 were originally developed for the following three traits by Dr. H.S. Dhaliwal and his coworkers at Punjab Agricultural University (PAU), Ludhiana, India: (i) grain protein content (GPC); (ii) preharvest sprouting tolerance (PHST), and (iii) grain weight (GW).

QTL analyses for 11 yield and yield-related traits. The GPC and ITMI populations were used to identify QTL for nine yield traits including plot yield and its components, plant height, and peduncle length. For this purpose, single-locus (using QTL Cartographer) and two-locus (using QTLNetwork) QTL analyses were conducted. For all 11 traits, a total of 80 putative M-QTL on 19 chromosomes in the GPC population and 140 putative M-QTL on 20 chromosomes in ITMI population were detected. QTLNetwork identified a total of 113 and 190 QTL that included QTL with significant main effect and/or significant interaction effect (epistatic QTL or QTL involved in interaction with the environment). An important genomic region harboring important major co-localized QTL for each of the six yield traits was identified on chromosome arm 2DS in both the GPC and ITMI populations. In the ITMI population, this QTL influenced 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, the QTL influenced 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 genomic region with the above QTL was physically located in the distal bin (2DS5-0.47-1.00) covering 53% region of 2DS. Comparative mapping revealed that the genomic region harboring the QTL in wheat spans a distance of 11.51 Mb on rice chromosome 7 (R7). This information may prove useful for high-resolution mapping leading to map-based cloning of the above major QTL.

Marker-assisted selection for GPC and leaf rust resistance. In bread wheat, high grain protein content (HGPC) determines nutritional value, processing properties, and quality of the end-product. In view of this, marker-assisted selection (MAS) was used to introgress a major gene for high GPC (*Gpc-B1*) into six wheat genotypes. These six wheat genotypes included (i) three elite Indian bread wheat cultivars and (ii) three advanced lines derived from the cultivar PBW343 (each containing the leaf rust resistant gene *Lr*24). During backcrossing, foreground selection was exercised using tightly linked markers. Background selection was performed using SSR markers evenly distributed throughout the genome. As a result, 14 BC_3F_4 lines carrying *Gpc-B1* were developed and evaluated for GPC and grain yield. Ten of