Fábián A, Jäger K, Darkó É, and Barnabás B. 2008. Cryopreservation of wheat (*Triticum aestivum* L.) egg cells by vitrification. Acta Physiol Plantarum 30(5):737-744.
Jäger K, Fábián A, and Barnabás B. 2008. Effect of water deficit and elevated temperature on pollen development of drought sensitive and tolerant winter wheat (*Triticum aestivum* L.) genotypes. Acta Biol Szeged 52(1):67-71.

ITEMS FROM INDIA

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Current activities: Improvement of wheat quality and rust resistance in Indian wheat.

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

Improvement of wheat for quality in an Indian wheat background is being carried out by using HMW-glutenin subunits as a selection criterion. The rust resistance genes *Sr31*/*Lr26*/*Yr9* and *Sr26*, *Sr24*/*Lr24* are being combined with high yielding ability and specific HMW subunits. Selected lines from several intervarietal crosses in different generations (F_2, F_3, and F_4) are being evaluated.

Radiation-induced mutations in wheat.

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

Earlier, the cultivar C306, known for its good chapati-making quality was treated with gamma rays, and mutants with early flowering were isolated in the M_2 generation. The parent showed anthesis in about 75 days, whereas the mutants showed anthesis in 50 to 63 days. Seven mutant lines were analysed for quality traits. Grain-protein content ranged from 11.9 to 14.9% compared to 13.1% in the parent. SDS-PAGE of total grain protein showed that the mutants had an unaltered HMW-glutenin subunit pattern. Rheological properties estimated using a Brabender Farinograph showed that the mutant lines had comparable water absorption, dough-development time, dough stability, degree of softening, and quality number. The early mutants are being carried forward.

MP3054 and Hindi 62 were treated earlier with gamma rays. M_2-generation plants were grown in 2008-09. Plants that flowered early and had reduced culm length were identified as mutants and harvested individually.

A bread wheat genetic stock with morphological markers for dark glumes, hairy glumes, hairy leaf, purple culm, and red grain was mutagenized with gamma rays. In the M_2 generation, plants with altered morphology were identified and individually harvested. The M_3 was grown as plant-to-row progeny. Although variations for the extent of glume pigmentation or hairiness, spike morphology, and culm length were observed, lines were found to segregate for the mutant traits.

Validation and marker-assisted selection for rust resistance and quality-related genes in Indian wheat.

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

Validation of SCAR marker $SCS1302_{609}$ for gene *Sr24*. Molecular markers developed for traits such as disease resistance using a specific genotype may not necessarily work in others. Hence, validating markers in diverse genotypes is important. In this study, marker $SCS1302_{609}$ (Gupta et al. 2006) reported for *Lr24*/*Sr24* was validated by analyzing

wheat genotypes/cultivars with wide genetic background and also in segregating populations. PCR conditions were optimized by gradient PCR at different temperatures (60.3°C, 61.1°C, 61.9°C, 62.3°C, and 63°C). The optimum annealing temperature was found to be at 61°C. Forty-one wheat genotypes were screened using the primers for SCAR marker $SCS1302_{609}$. The genotypes with *Sr24* yielded a 607-bp band. Wheat genotypes that were reported to carry other *Sr* genes, i.e., *Sr31*, *Sr26,* and also noncarriers of *Sr24^,* did not amplify this marker, indicating that SCAR marker $SCS1309_{609}$ was specific only to gene *Sr24* in the Indian wheat genotypes/cultivars.

SCAR marker analysis in segregating populations. The SCAR marker $SCS1302_{609}$ also was validated by analyzing two segregating populations (Kalyansona/Vaishali and Kalyansona/Vidisha). The genotypes (RR, Rr, and rr) of individual plants in the F_2 generation were identified by scoring the rust reaction of respective F_3 progenies. Analysis of DNA from these plants using marker $SCS1302_{609}$ showed that, out of the 52 resistant plants, 51 amplified the SCAR marker and one failed to amplify. Of the 21 susceptible plants, 19 did not amplify the marker and two showed amplification. This result deviated from the expected 9:3:3:1 (Res/+:Res/–:Sus/+:Sus/–) ratio for independent assortment between the stem rust-resistance locus *Sr24* and the SCAR marker. Three recombinants were observed in the F_2 population. Using MAPMAKER (version 3.0), the distance between $SCS1302_{609}$ and the *Sr24* locus was estimated to be 4.3 cM.

Similarly, an F_2 population from the cross 'Kalyansona/Vidisha' was screened for rust reaction and the presence of marker $SCS1302_{609}$. A total of 18 plants were screened in the F_2 generation for their phenotype. Of the 14 F_2 plants with a resistant phenotype, five were confirmed to be homozygous and nine were confirmed to be heterozygous based on the phenotypes of their progenies in the F_3 generation, and four F_2 plants were found to be homozygous for a susceptible reaction. All the resistant plants showed amplification of the SCAR marker. All five susceptible plants did not amplify this marker. The marker, therefore, was found to be suitable for screening *Sr24* in early generation material.

Marker-assisted breeding to combe rust resistance genes *Sr24* and *Sr31* and *Glu-D1d* (coding for HMW-glutenin subunits 5+10) is underway in a cross between FLW-2 and Kite. In the F_2 generation, ~220 plants are being analyzed using SCAR markers. Plants with both rust-resistance genes and *Glu-D1d* will be selected and advanced.

Marker-assisted backcrossing. To transfer *Sr24* and *Glu-D1d* into HD2189, marker-assisted backcross breeding (MAB) is being carried out. Thirty BC_3F_1 plants were grown, and DNA from leaves of 4-week-old individual plants was extracted and screened using SCAR markers for the two genes. In the winter of 2008–09, seven plants with both markers were identified. Backcrosses were made using the HD2189 recurrent parent and carriers of both the markers.

Genotyping of an RIL population for variation at the Xgwm261 locus.

S. Bakshi and S.G. Bhagwat.

A 192-bp allele at the *Xgwm261* microsatellite locus is known for its association with reduced height gene *Rht8* in hexaploid wheat. Indian wheat cultivars showed a predominance of 165-bp, 174-bp, and 192-bp alleles at this locus. In our earlier analysis of Indian wheat cultivars, the 192-bp allele at the *Xgwm261* locus did not show association with height reduction at the Trombay location, which is a warm environment. An RIL population of 139 lines derived from the two cultivars Sonalika (165 bp) and Kalyansona (192 bp) was assayed for polymorphism at the *Xgwm261* locus. The RIL segregation fit a 1:1 ratio for the presence of 165-bp and 192-bp alleles. These RILs were grown in the field at Trombay during the winter of 2008–09, and data for phenotypic traits of culm length (cm), spike length, spikelet number, and flag-leaf blade area (cm) were recorded. Plant growth was affected by heat stress during the season. Further analysis is in progress.

Genetic relationships among bread wheat genotypes with different seedling thermotolerance using parentage analysis, SSRs, and agronomic data.

Heat stress affects the productivity of wheat in many wheat-growing regions of India. The tolerance of wheat plants to higher than optimum temperature varies at different plant-growth stages. Seedling thermotolerance was assessed among 56 genotypes using membrane thermostability (MTS) and triphenyl tetrachloride tests (TTC). Twenty genotypes with varying thermotolerance were selected and grown in heat stressed and non-heat stressed environments to evaluate their phenotypic performance. Parentage data were used to find the degree of relationship among these genotypes. The

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.

C. Chang, P. Suprasanna, and S.G. Bhagwat.

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.

Publications.

Bhagwat SG, Sud S, and Das BK. 2007. Radiation induced mutations for crop genetics and improvement. In: Isotopes Applications in Agriculture. IANCAS Bull VI(4):293-298.

Das BK and Bhagwat SG. 2008. Isolation of early flowering mutant in cultivar C-306 known for its good *Chapati* making quality. In: FAO/IAEA Internat Symp on Induced Mutations in Plants. 12-15 August, 2008, Vienna, Austria. Book of Abstracts, pp. 155-156.

Das BK and Bhagwat SG. 2009. AP-PCR analysis of Indian wheat genotypes: Genetic relationships and association analysis. Wheat Inf Serv (http://www.shigen.nig.ac.jp/ewis/article/html/41/article.html;jsessionid=7FB268AEEDE536CF27095EB459C487BB.4_5).

Das BK, Saini A, Bhagwat SG, and Jawali N. 2006. Marker assisted selection for stem rust resistance gene *Sr24* in Indian wheat genotypes: Validation of a SCAR marker. J Genet Breed 60:189-196.

Sud S, Nayeem KA, and Bhagwat SG. 2008. Molecular genotyping of GA3 insensitive reduced height mutant of emmer wheat (*Triticum dicoccum*). In: FAO/IAEA Internat Symp on Induced Mutations in Plants. 12-15 August, 2008, Vienna, Austria. Book of Abstracts, p. 190.

Saini A, Das BK, Bhagwat SG, and Jawali N. 2008. Rapid identification of a hidden co-migratory AP-PCR marker in wheat by band-stab PCR-RFLP. Ann Wheat Newslet 54:54-56.

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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