

genotypes, but other cultivated cereals (barley, triticale, and durum wheat) also are represented. The breeder collection is an important part of the cereal breeding program at Martonvásár.

Genetic stock collection. Special attention is given to the high-value genetic stocks (1,000 accessions), such as aneuploid material (nullisomic, monosomic, substitution, and addition series), special mutant stocks, and amphiploids. A majority of the genetic stocks were developed at Martonvásár during the last few decades, e.g., the Rannaya 12 monosomic series, and the other part was collected via international material exchanges. The genetic stock collection is used in basic genetic research and prebreeding activities. The maintenance of genetic stocks often requires cytogenetic control.

Wild wheat relatives. This collection consists of about 1,700 accessions, including a majority of species of *Triticum*, *Aegilops*, *Secale*, *Hordeum*, and perennial species of *Agropyron* and *Elymus*. Many of these accessions possess excellent resistance for biotic and abiotic stresses. They are used mostly in prebreeding work. Several prebreeding programs were started at Martonvásár on the basis of genebank accessions to transfer useful traits of wild relatives in to hexaploid wheat. The *T. monococcum* collection, with more than 300 accessions, is outstanding among the European collections. The *Aegilops* collection has expanded in recent years by collecting 130 new accessions via European expeditions.

The entire genebank collection consists of around 14,000 accessions. The long-term, *ex situ* maintenance of the accessions take place in refrigerated storage (-28°C). The majority of the genebank accessions are stored for medium term at 4°C in a cold room. At the same time, perennial species also are maintained *in situ* in an isolated nursery.

Database management of the genebank uses the *Breeder* software, which was developed at Martonvásár for cereal breeding. Characterization of genebank accessions under field conditions is an important part of our activity. All phenotypic and agronomic data collected during the regeneration and conservation processes are recorded in the *Breeder* database, which also is used to manage seed production, storage, and exchange.

ITEMS FROM INDIA

BHABHA ATOMIC RESEARCH CENTRE

Nuclear Agriculture & Biotechnology Division, Mumbai-400085, India.

Development of a gamma ray-induced mutant line in the wheat cultivar PBW-343 with moderate resistance to wheat stem rust race Ug99.

B.K. Das, Vikas, G. Vishwakarma, and S.G. Bhagwat.

Stem rust is a deleterious disease of wheat. In the recent past, the appearance of virulent races, such as Ug99, have broken many important stem rust resistance genes in wheat and barley. Under the aegis of an IAEA project (INT5150, 'Responding to the Trans-boundary Threat of Wheat Black Stem Rust (Ug99)'), the wheat cultivar PBW-343 was irradiated with gamma rays (250, 300, and 350 Gy). The M_1 was raised at Trombay in 2009–10. The M_2 population was sent to a hot-spot for Ug99 in Kenya in 2011 for screening for resistant mutants. Mutants were identified as moderately resistant (MR) or moderately susceptible (MS). These mutants were carried forward to the M_3 and M_4 generations in Kenya. Seed of one mutant line having an MR reaction were brought back to India, and this mutant line (TWM-97) is being multiplied at our station. Morphological characters were noted and molecular characterization of the mutant line is being carried out. Crosses were made with the parent cultivar for an allelism study. We are thankful to Drs. P. J. L. Lagoda and T. Moleah (IAEA) for supporting this program and Prof. M. Kinyua (Eldoret, Kenya) for screening for Ug99 resistance.

Development of an early maturing, mutant line in the wheat genotype MP-3054.

Vikas, G. Vishwakarma, A.S. Shitre, S. Bakshi, S.G. Bhagwat, G.A. Gadekar (Agriculture Research Station, Niphad, Maharashtra), and B.K. Das.

Due to changes in climate, particularly the rise in atmospheric temperature, wheat crop yields have been affected. Heat stress during the seedling, grain filling, and maturity stages are important, reducing the overall productivity. Wheat genotypes with longer duration to maturity suffer from terminal heat stress. Wheat genotype MP-3054 has moderate tolerance to heat stress, taking nearly 70 days to flower and 98 days to mature. Using gamma ray-induced mutation, we have developed early mutant lines (TWM-93 and TWM-94) that are 14-19 days earlier than the parent genotype. The mutant lines flower in 51–56 days and mature in 79–98 days. These mutants now are in the M_0 generation and stable. These lines are being evaluated at Trombay, Niphad, and Akola. This work was carried out under an IAEA-RCA project RAS5056.

Induced-mutation approach for improvement of yellow rust resistance in Indian wheat.

G. Vishwakarma, Vikas, A. Shitre, and B.K. Das.

Rust is a very deleterious disease in wheat. Among the three rusts (black, brown, and yellow), yellow, or stripe, rust is more prevalent in the North-West Plain Zone and Northern Hill regions of India. In the recent past, due to the appearance of many virulent races of stripe rust, many newly released, high-yielding cultivars carrying important resistance genes to stripe rust have become susceptible. Therefore, we have initiated an induced-mutation approach for genetic improvement of yellow rust resistance in Indian wheat. We already have irradiated a few of the recently released wheat cultivars with gamma rays, and the M_1 was grown in Trombay. M_2 seeds were obtained and will be screened in a stripe rust infection hotspot and subsequent generations in laboratory and glass house conditions. This work is being carried out in collaboration with ICAR–IIWBR, Karnal; ICAR–IIWBR Regional Station, Flowerdale; and the G.B. Pant University of Agriculture & Technology, Pantnagar.

Induced-mutation approach for genetic improvement of agronomic traits in the wheat cultivars HI-1500 and HI-1531.

G. Vishwakarma, V. Sai Prasad (IARI Regional Station, Indore, Madhya Pradesh), Vikas, and B.K. Das.

Wheat cultivars with maturity times of long duration are affected by terminal heat stress. The wheat cultivars HI-1500 and HI-1531 are popular as check cultivars and widely cultivated in the Central zone of India. Due to the long duration in maturity, the cultivars are affected by heat stress. In order to reduce the maturity period and height, an induced-mutation approach was followed. The two cultivars were irradiated with gamma rays, and the M_1 population was raised at Trombay. The M_2 population will be screened in the coming year. This work is being carried out in collaboration with the ICAR–IARI Regional Station, Indore.

A study of the inheritance and molecular characterization of an early maturing, mutant line TWM-89-2 in wheat cultivar C-306.

G. Vishwakarma, A. Saini (Molecular Biology Division, BARC, Mumbai), Vilkas, A. Shitre, and B.K. Das.

Using gamma rays, we developed an early flowering and maturing mutant line in the wheat cultivar C-306 (under an IAEA-RCA project RAS5045). This line (TWM-89-2) has been deposited at the National Bureau for Plant Genetic Resources, New Delhi, with national id number IC0611305. Molecular markers (AP-PCR, STMS, and AFLP) are being used to characterize the mutant line and study polymorphism between the parent and the mutant line. In order to study the inheritance of the early maturing trait and develop molecular markers, crosses were made between the mutant and the parental cultivar. The F_1 seeds were grown, and F_2 seeds obtained. An F_2 mapping population will be used for studying genetics and developing linked marker(s).

Inducing mutations in Indian wheat using physical mutagens, electron, proton, and ion beams.

Vikas, G. Vishwakarma, A. Shitre, and B.K. Das.

In India, gamma rays are used most commonly for inducing mutations for crop improvement. However, by using other physical mutagens, such as electron, proton, or ion beams, many mutants were obtained in other countries. In order to explore the use of these mutagens, we have standardized the dose and other technical parameters in collaboration with the Raja Ramanna Centre for Advanced Technology, Indore (Dr. V.C. Petwal) and the BARC Pelletron Facility at TIFR, Mumbai (Drs. J.P. Nair and A.K. Gupta). Electron beam irradiation of three wheat cultivars was done and the GR50 determined. Technical standardization for irradiation of wheat seeds with proton and ion beams is in progress.

Validation of SCAR markers for leaf rust resistance gene Lr32 in Indian wheat genotypes.

G. Vishwakarma, Vikas, and B.K. Das.

The leaf rust resistance gene *Lr32* has not yet been fully exploited in Indian wheat breeding programs. In order to use this gene in breeding and for pyramiding with other *Lr* genes, molecular markers linked to this gene will be very useful and efficient. A SCAR marker for this gene was reported by in 2010. Our aim was to validate this marker in Indian wheat genotypes and a segregating population. PCR amplification was done in five genotypes with *Lr32* and 10 genotypes without *Lr32*. A 350-bp band was amplified in genotypes with *Lr32*, which was absent in noncarriers. For further confirmation, we are analyzing a segregating population from the cross 'Agra Local / TC+*Lr32*'.

Validation of SCAR marker for Glu-D1d (coding for HMW-glutenin subunits 5+10) in Indian wheat genotypes.

G. Vishwakarma, Vikas, and B.K. Das.

The HMW-glutenin subunits 5+10 (coded by *Glu-D1d*) are known to influence dough strength and bread-making quality. In our wheat breeding program, these subunits are selected using SDS-PAGE. However, a marker-assisted breeding program will ease the process. A SCAR marker (478 bp) is linked to this gene. We validated this marker in Indian wheat genotypes. The carriers amplified a 478-bp band that was not amplified in noncarriers. This marker is more robust than the 450-bp marker reported by D'Ovidio and Anderson (1994). For further confirmation, we are using a segregating population from the cross 'Kalyansona (*Glu-D1a*; coding for subunits 2+12) / PBW-343 (*Glu-D1d*; coding for subunits 5+10)'.

Improvement of wheat cultivar HD-2189 by incorporating rust resistance genes Sr24/Lr24 and HMW-glutenin subunits 5+10 (Glu-D1d) by marker-assisted backcross breeding.

S.G. Bhagwat, A. Shitre, G. Vishwakarma, Vikas, G.A. Gadekar (Agriculture Research Station, Niphad, Maharashtra), and B.K. Das.

The wheat cultivar HD-2189 is popular in the Peninsular zone of India. For improvement of rust resistance and dough strength, two genes, *Sr24/Lr24* and *Glu-D1d* (coding for HMW-glutenin subunits 5+10), were incorporated from the genetic stock KS-3 (developed at our centre). Marker-assisted backcross breeding was carried out. The lines are now in the BC₅F₄ generation and are being evaluated for yield and other characteristics. We are proposing for a yield trial under special marker-assisted backcross breeding lines.

Developing low-cost, high-throughput screening of SCAR and STMS markers in wheat using SYBR green-based, low-resolution melt profiling.

G. Vishwakarma, S.G. Bhagwat, and B.K. Das; and A. Saini, R. Sanyal, and N. Jawali (Molecular Biology Division, Bhabha Atomic Research Centre, Mumbai-400085, India).

PCR-based, single-locus, DNA markers, such as SCAR and STMS markers, commonly are used in crop breeding experiments for diverse applications. These markers are generally scored using agarose or polyacrylamide gel electrophoresis. However, these techniques are laborious, time consuming, and not cost-effective for analyzing large populations. Low-resolution melt profiling, based on low cost dyes (such as SYBR green), could be effectively utilized for detecting single-locus, PCR markers (SCAR or STMS) in a gel-free manner. The feasibility of the approach was demonstrated using SYBR green-based, melt profiling in a few STMS and SCAR markers in ten bread wheat genotypes. Clean melt curve profiles were obtained for all markers with no background noise from unused template DNA or primer dimers. Individual STMS and SCAR markers could be detected easily in different genotypes. In addition, polymorphic STMS markers also could be identified in melt curve profiles. This approach is ~20% cheaper than the conventional, gel-based methods. Furthermore, the possibility of multiplexing and reducing the volume of reaction mix provides further scope for reducing the cost of screening. Screening STMS and SCAR markers by this high-throughput method will be useful in estimating linkage and gene mapping, genetic diversity and phylogenetic analyses, and marker-assisted selection. Although the current study was based on analysis of wheat samples, the method may be applicable to other crop plants.

Understanding the molecular mechanism of stem rust resistance genes in wheat.

G. Vishwakarma, A. Saini, N. Jawali (Molecular Biology Division, BARC, Mumbai), and B.K. Das.

Wheat production is affected by many biotic stress, of which stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is a potential threat worldwide and can cause 100% damage to yield. Understanding the molecular mechanism involved in resistance to stem rust is of utmost importance for achieving durable resistance against this disease. Two wheat genotypes, C-306 and Unnath C-306, hereafter UC-306 and C-306+*Sr24*, respectively, were injected with the *Pgt* pathotype 7G-11. A microarray analysis of the wheat transcriptome was done at three time points, 0, 10, and 72 hours post inoculation (hpi). Microarray results showed that before inoculation (0 hpi), a minimal number of genes were differentially regulated. However, at 10 hpi, approximately 300 genes were up-regulated in UC-306, indicating a major defense response against the pathogen. At 72 hpi, the transcriptome was comparable to that before inoculation. Differentially regulated transcripts were annotated using the DFCI gene indices database. Transcripts related to pathogenesis related proteins, phytoalexins, transcription factors, biotic stress, cell membrane transporters, kinases, and xylan synthase were found to be up regulated in the resistant cultivar and, hence, are key players in resistance to stem rust pathogen. Validation of transcription levels of a few important transcripts is being carried out using quantitative RT-PCR. This work is being carried out in collaboration with ICAR-IIWBR RS, Flowerdale, and the IARI Regional Station, Wellington.

Genetic variability of phytic acid and inorganic phosphorus in diverse wheat germplasm.

Suman Bakshi, Vikas Kumar, Abhijeet Shitre, and B.K. Das.

Phytic acid (myo-inositol hexakisphosphate), the major storage form of phosphorus in seeds, is believed to have a negative impact on nutritional quality. The phytic acid concentration reported in wheat germ and bran are 1.1–3.9% and 2.0–5.3%, respectively. More than half of the world population is affected by micronutrient malnutrition, and one-third of the world's population suffers from anemia and zinc deficiency, particularly in developing countries. Iron and zinc deficiencies are major health problems worldwide. Phytic acid chelates micronutrients and prevents their bioavailability in monogastric animals, including humans, because they lack the enzyme phytase in their digestive tract. Several methods have been developed to reduce the phytic acid content in food to improve its nutritional value, including the genetic improvement of food grain and several pretreatment methods. Because breeding for low phytic acid has been proposed for several cereals and legumes, it is important to evaluate genetic variation for phytic acid content among the available germplasm. Colorimetric estimation was done for both phytic acid and inorganic phosphate in 270 diverse wheat genotypes. Significant genotypic variation was found among the tested genotypes. The range for phytic acid content is 4.48 to

14.8 mg/g with a mean of 10.56 mg/g. The range for inorganic phosphate content is 0.30 to 1.8 mg/g. Wheat germplasm is not extensively evaluated for genetic variation for phytic acid. Screening of large diverse germplasm available indicated that phytic acid is a highly heritable trait ($h^2 = 0.76$) and selection for low phytic acid is possible.

Analysis of advance wheat genotypes of peninsular zone of India for phytic acid, inorganic phosphate, iron and zinc content.

A.S. Shitre, G.A. Gadekar (Agriculture Research Station, Niphad), V. Ramachandran, Vikas, and B.K. Das.

Malnutrition (particularly iron and zinc) is a common problem worldwide, more severe in developing countries. Bio-fortification and some other measures are available, but they are difficult to implement and use in day-to-day activities. One suggested method is genetic improvement of cereals with increased iron and zinc content and reduced phytic acid content, which binds with these minerals and makes them unavailable for digestion in nonruminants. One hundred advanced breeding lines of wheat, developed for the Peninsular zone of India, were analyzed for iron, zinc, phytic acid, and inorganic phosphate content and significant variability was observed. Iron content was 0.042–0.098 mg/g and zinc content was 0.017–0.029 mg/g. Phytic acid content ranged from 4.97 mg/g to 15.02 mg/g (mean of 9.58 mg/g). Inorganic phosphate content was ranged from 0.128 to 0.234 mg/g of seed. The parameters exhibiting higher estimates of GCV, coupled with high heritability, indicate that considerable improvement in these parameters can be achieved through incorporating desired genotypes in a crossing program followed by pedigree selection.

News from our wheat research group.

Mr. G. Vishwakarma received the 'Best Poster' award for the presentation 'Low resolution SYBR green dye based melt profiling for analysis of STMS and SCAR markers in plants' during the National Symposium on Crop Improvement for Inclusive Sustainable Development held at Ludhiana, India, 7–9 November, 2014.

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