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Volume 37

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Jules Janick

Purdue University

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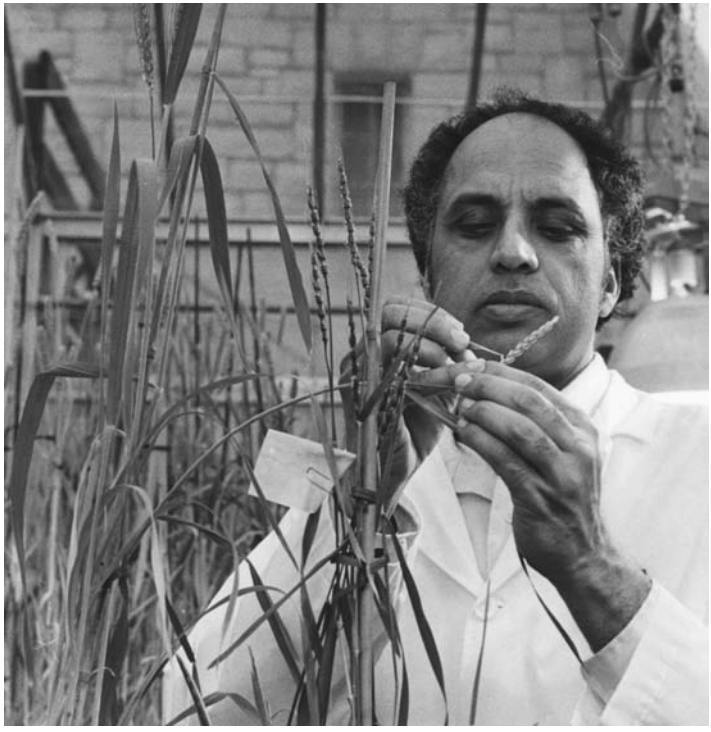

Bikram Gill

1

Bikram Gill: Cytogeneticist and Wheat Man

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Bikram Gill's brothers proudly refer to him as their "Wheat Man" and, in fact, the Kansas Association of Wheat Growers named him the "Wheat Man of the Year" in 1997. His numerous awards for his wheat research attest to this fame (Table 1.1). Bikram had always wanted to be a scientist of the type who helps people make the world better. Although he thought his botanical training in India was a waste of time, he now knows better. It was his mentor Charlie Rick who taught him that using science to understand nature and serve society is both exciting and rewarding. Nearly five decades later that commitment has helped his dream become reality. Bikram has won more than $20 million in extramural grants to support his research, including significant funding from the Kansas Wheat Commission and the USDA for establishing a gene bank at Kansas State University and wheat genetics research, the McKnight Foundation for Fusarium head blight research, and the National Science Foundation and USDA for wheat genome sequencing. Bikram is the author or coauthor of more than 350 refereed journal publications, 230 abstracts, 17 book chapters, and 54 newsletter items. He has contributed papers to more than 60 conference proceedings and partnered in the release of 54 germplasm lines. He has presented more than 200 lectures both nationally and internationally. He is the coauthor of *Chromosome Biology*. Volume 37 of *Plant Breeding Reviews* is dedicated to Bikram Gill's illustrious and extraordinary career. (A complete list of publications of Bikram Gill is available at http://www.k-state.edu/wgrc/Publications/pubstoc.html)

I. EARLY LIFE: EMERGENCE OF A CYTOGENETICIST

Bikram S. Gill was born on 31 October 1943, in a small village called Dhudike, District Moga, Punjab, India. He was the fifth of 10 children. His parents were farmers; his father also served as a *lambardar* (revenue collector) and a *sarpanch* (mayor) of the village. Bikram was always very interested in education and worked hard on his homework, graduating from high school in 1957 first in his class. He studied at DM College at Moga as a premedical student from 1959 to 1961. Bikram then went on to earn his B.S. degree at Khalsa College, Amritsar, in 1963, followed by B.S. Honors and M.S. Honors degrees in 1966 from Punjab University at Chandigarh where he became really interested in botany. Bikram lectured premedical students at GHG Khalsa College, Gurusar Sudhar, from 1966 to 1968.

In 1968, he was admitted to Brigham Young University where his brother, Gurcharan, was teaching mathematics. His brother had tried to

Table 1.1. Awards, honors, and service of Bikram Gill.

Phi Kappa Phi Award for Academic Excellence, University of California, Davis, 1973
D.F. Jones Postdoctoral Fellowship, University of Missouri, Columbia, 1973–1974
Visiting Professor, CSIRO, Division of Plant Industry, Canberra, Australia, 1986–1987
Visiting Professorship to the German Democratic Republic, U.S. National Academy of Sciences, 1987
International Organizing Committee, Wheat Genetics Symposium, 1988–1998
Chair, International Committee on Wheat Chromosome Banding Nomenclature, 1988–1998
Editorial Board, *Plant Breeding*, 1990
Conoco Distinguished Graduate Faculty Award, Kansas State University, 1990
Elected Fellow, American Society of Agronomy, 1991
Visiting Scholar to India, UNESCO-TOKTEN, 1991
Visiting Professor to Russia and Ukraine, U.S. National Academy of Sciences, 1992
UNDP Visiting Scholar, People's Republic of China, 1993
Board of Directors, Crop Science Society of America, 1994
Editorial board, *Crop Science*, 1994
Elected Fellow, Crop Science Society of America, 1994
Associate editor, *Theoretical and Applied Genetics*, 1995
Visiting Professor, Ludwig Maximillian University, Munich, Germany (DAAD Fellow, Germany–U.S. Exchange Program). 1995–1996
University Distinguished Professor, Kansas State University, 1997
Higuchi Research Achievement Award/Irvin E. Youngberg Award in the Applied Sciences, University of Kansas, 1997
Wheat Man of the Year, Kansas Association of Wheat Growers, 1997
Editorial board, *Genetics*, 1998
Fellow, American Phytopathological Society, 1998
Crop Science Research Award, Crop Science Society of America, 1998
Outstanding Scientist Award, American Association of Agricultural Scientists of Indian Origin, 1999
Listed in the "Century's Top 10 Sikh Scientists," Panj Darya magazine, Punjab, India, 1999
Fellow, American Association for the Advancement of Science, 1999
Fellow, National Academy of Agricultural Sciences of India, 2001
Listed among the world's top highly cited scientists in Animal and Plant Sciences by Thompson Reuters, 2006
Foreign Fellow, National Academy of Sciences, India, 2006
International PI, 111 Project, "Crop Genetics and Germplasm Enhancement," Nanjing Agricultural University, 2008
Friendship Medal, Jiangsu Province, Nanjing, China, 2010
Editorial board, *G3: Genes|Genomes|Genetics*, 2011
Frank N. Meyer Medal for Plant Genetic Resources, Crop Science Society of America, 2011
Editorial Board, Agricultural Research, Official publication of the National Academy of Agricultural Sciences, India, 2012
National Friendship Award, Government of China, Beijing, 2012

talk him into working toward being a medical doctor, but he insisted on botany because of his dream of feeding the world. Working with Howard Stutz at Brigham Young, Bikram developed a chromosome staining technique for cereals that impressed Ralph Anderson very much. Ralph had studied with Charlie Rick at the University of California (UC), Davis, and advised Bikram that was where he needed to be. Bikram began his graduate work with Charlie in 1969. His Ph.D. thesis was on the cytogenetics of tertiary aneuploids with unusual transmission characteristics in tomato. When he came to Kansas State, he was frequently seen wearing a green fishing cap, Charlie's trademark.

After graduating from UC Davis, Bikram moved to the University of Missouri. As a graduate student, he had written a grant proposal for chromosome banding in wheat. Bikram had read about the work that was being done in human cytogenetics with chromosome banding and wanted to achieve the same in wheat. At Missouri, Bikram had the opportunity to work with the late Ernie Sears and Gordon Kimber. During that time, he was introduced to David Apirion from Washington University in St. Louis, and switched to mouse molecular biology. But it was just for a year, because then, following his heart, he was back into wheat research with Giles Waines at the University of California, Riverside. At Riverside, Bikram met Lennart Johnson, an avid collector and researcher of wild wheat species, who introduced him to the world of genetic resources.

On his birthday 31 October 1977, Bikram received a call from Don Meyers, Director of the AREC at the University of Florida, Belle Glade, that he had been hired as an assistant professor to work on sugarcane genetics and breeding. He can still recall that on the first day of work there, he was going out in the field to make selections on a crop he had yet to set eyes on. Thinking he had found his niche with sugarcane, Bikram was surprised when one day he received a call from Gordon Kimber, who told him that Kansas State was looking for a wheat cytogeneticist and that he had already submitted his CV for the position. Barely a year and a half later, he was on his way to Manhattan, Kansas. The world of wheat had called him back, and this time he would not leave.

II. RESEARCH

A. Tomato Cytogenetics in California

Gurdev Khush had left UC Davis to become the rice breeder at the International Rice Research Institute in 1968. Later, he would earn

world acclaim as one of the fathers of rice revolution. Bikram filled his slot and learned tomato cytology from him when he made a return visit to Davis in the summer of 1969. Bikram immensely enjoyed his graduate studies at UC Davis and became steeped in tomato cytogenetics research with Charlie Rick (Gill 1983). Charlie mapped the tomato genome, collected wild tomatoes on his frequent explorations to South America, and all his students participated in tomato genetics and wide-hybridization research, in addition to their specific projects. Graduate students shared coauthorship for participating in the on-going research (Rick and Gill 1973) but were solo authors on publications from their thesis research (Gill 1974a,b). Bikram's expertise in tomato cytology won him his first NSF award at K-State in 1980 to study cytogenetic basis of somaclonal variation in potato with similar chromosome morphology. As he was finishing up his graduate studies at UC Davis, Bikram submitted a solo, two-page project to the Research Corporation in New York. Charlie wrote to E.R. Sears at the University of Missouri to sponsor laboratory space.

B. Chromosome Banding Research in Missouri

Chromosomes are characterized by their length, arm ratio, and the presence or absence of secondary constrictions. Conventional staining techniques did not distinguish between morphologically similar chromosomes, so techniques that allowed fast and reliable identification of chromosomes were needed. Bikram's proposal, submitted to Research Corporation in New York, which was founded by D.F. Jones from his patent on hybrid corn, was funded for this research. In the summer of 1973, Bikram began working at the University of Missouri, Columbia, in the laboratory of Gordon Kimber (it turned out that Ernie Sears did all of his work in his office, so all visitors to Missouri worked in Gordon's laboratory. The research was wildly successful (Fig. 1.1); wheat and rye chromosome could be cytogenetically identified for the first time and their chromatin differentiation into euchromatin and heterochromatin states could be rapidly determined (Gill and Kimber 1974a,b).

In the summer of 1974, Bikram attended meetings of the International Congress of Genetics at UC Berkeley followed by a vacation. Upon returning, he was shocked to discover that he could not reproduce the work for which he had published two high-profile papers! Eventually, after recreating his work schedule, Bikram realized that after making chromosome preparations, he used to leave the slides in ethanol and go home for lunch. His new schedule did not include a

Fig. 1.1. Bikram at work in Missouri, 1973, soon after the discovery of the heterochromatic banding patterns of wheat chromosomes. Everyone in the legendary Curtis Hall came to take a peek under the microscope.

lunch break, and so the long, ethanol treatment was skipped. To his surprise, and delight, chromosome spreads dehydrated in ethanol for a long time showed the most beautiful and high-contrast banding patterns he had ever seen. So, just by accident, Bikram had discovered the importance of dehydrating chromosome spreads before C-banding to obtain differentially stained chromosomes (Gill and Kimber 1974a,b).

C. Wild Wheat Studies at UC Riverside

While Bikram was at Missouri, rumors were rife that Lennart Johnson and his student Harcharan Dhaliwal at UC Riverside had discovered the elusive B-genome donor of wheat. Bikram called Harcharan and got a few seeds of this new species with the unfamiliar name *Triticum urartu*. Bikram immediately profiled the *T. urartu* chromosomes with the new staining technique. They were unlike the B-genome and more similar to the A-genome chromosomes of wheat. Bikram let Harcharan know about these results and soon other labs provided evidence of the homology between *T. urartu* and the A genome of polyploid wheat. In fact, Harcharan's own data pointed to the same conclusion, but Johnson would not hear it. Later, molecular work would provide

Fig. 1.2. The WGRC welcomed visits from many prominent wheat scientists throughout the years, such as this group in 1989: (L to R, front row) Bernd Friebe, Ernie Sears, Takashi Endo, and Yashuiko Mukai; (back row) John Raupp, Christine Curtis, Adam Lukaszewski, Bikram Gill, and Bali Ram Tyagi.

conclusive evidence that *T. urartu* was, in fact, the A-genome donor of polyploid wheat. Bikram spent two years at UC Riverside studying wild wheat species and especially endosperm development in wild wheat species hybrids. In due course, Ernie's cytogenetic stocks (Fig. 1.2) and Johnson's wild wheat collection would form the foundation material to launch his career at KSU.

D. Sugarcane Breeding in Florida

Although Bikram stayed in Florida for only a year and a half (January 1978 to May 1979), he immersed himself into sugarcane cytogenetics and breeding research. USDA geneticist C.O. Grassl had assembled a world collection of sugarcane species and had made many intergeneric hybrids at Canal Point, on the banks of Lake Okeechobee. Bikram analyzed the chromosome constitution of these hybrids and published what he considers is an important paper in wide-hybridization research (Gill and Grassl 1986). He traveled to Minnesota to observe *in situ* hybridization research in Ron Phillips' laboratory and to work on a joint manuscript with Charlie Burnham on the development of a tester set of translocations for the tomato genome (Gill et al. 1980). He also submitted a grant proposal on sugarcane cytogenetics to the USDA, which although not funded was well reviewed.

E. Germplasm Evaluation and Enhancement in Kansas

Early on, Bikram realized that the wild relatives and related species are an important reservoir of agronomically interesting genes that can be used in wheat improvement and, in 1979, brought with him a part of the L.B. Johnson collection housed at the University of California, Riverside. At Kansas State, Jimmy Hatchett, a USDA–ARS entomologist, and coworkers already were looking at some synthetic wheats from the Kyoto University gene bank for resistance to a new biotype of Hessian fly, which was the most virulent isolate known at that time. They noticed that one particular line, derived from *Aegilops tauschii*, was highly resistant to Hessian fly (Hatchett et al. 1981). Bikram had 20 additional *Ae. tauschii* lines and, working with Hatchett, found five that were completely resistant to the new biotype (Hatchett and Gill 1981). Genetic analysis revealed that all carried different genes (Hatchett and Gill 1983). This small project was the beginning of a wide-hybridization program that would grow to encompass not only Hessian fly, but many plant diseases and traits as well.

Hari Sharma, a postdoctoral fellow whom Bikram met in Riverside, and John Raupp, the first research assistant, joined Bikram in 1980. Using embryo rescue, Bikram and Hari expanded the range of hybridization of wheat to certain perennial grasses and won Bikram his first USDA competitive grant in 1982. Together with Hatchett, Bikram's team grew to include Lewis Browder, a USDA scientist in Manhattan working with leaf rust; Tom Harvey, a K-State entomologist at Ft. Hays screening for greenbug; and John Moseman, a USDA scientist at Beltsville, Maryland, looking at powdery mildew resistance. The gene bank at Kansas State quickly grew during these initial years and by 1982, the collection contained 1867 lines of wild wheat and in three years had become one of the largest in the United States. From the start, Bikram envisioned the gene bank to be a working collection; extensively evaluated for disease and pest resistance and forming the basis for developing new germplasm for wheat breeders worldwide. The first of several germplasm evaluation papers was published (Gill et al. 1986).

Bikram's vision of a "one-stop shop" for wheat research became reality when he established the Wheat Genetics Resource Center (WGRC) at Kansas State University in 1984. The WGRC has been continuously supported by Kansas wheat growers through Kansas Wheat Commission grants since 1981 and USDA since 1989. Recognized as a center for excellence in wheat research by Kansas Board of Regents in 1984, the WGRC brought together plant pathologists,

entomologists, breeders, and USDA personnel with a vision of germplasm conservation and utilization for crop improvement for sustainable production by broadening the crop genetic base; creating and promoting the free exchange of materials, technology, and new knowledge in genetics and biotechnology among the world's public and private organizations; and sponsoring graduate and postgraduate students and visiting scientists for academic training and advanced research in the WGRC laboratories. The WGRC gene bank maintains accessions of all the wild wheat species and, in addition, cytogenetic stocks, the genetic treasures produced by a lifetime of work by wheat scientists. The WGRC established a national and international network to conduct and coordinate genetic studies in wheat. Genes for host–plant resistance to viral, bacterial, fungal, and insect pests and abiotic stresses would be identified, transferred to agronomically useful breeding lines, and deployed. State-of-the-art laboratories, greenhouses, and field plot facilities for teaching and research helped establish the WGRC.

Concurrently with the screening studies, Bikram initiated a direct hybridization program realizing that *Ae. tauschii* was readily amenable to crossing with wheat. Material testing resistant was crossed with wheat, embryo rescued, and segregating progenies produced. Homozygous, resistant germplasm lines could be produced in four generations (Gill and Raupp 1987). *Ae. tauschii* proved to be a goldmine for genes conferring resistance to diseases and pests. In 1984, Stan Cox was hired by the USDA as a plant geneticist and was the needed stimulus for the germplasm enhancement project. Stan helped arrange for the first germplasm releases from the WGRC, which were backcross lines developed by Bikram and Jim Hatchett that carried genes from *Ae. tauschii* for Hessian fly and soil-borne mosaic virus resistance in a Wichita background.

Bikram and John had found that, although the direct cross *Triticum aestivum* (AABBDD)/*Ae. tauschii* (DD) produced completely male-sterile ABDD F_1 plants, only one or two backcrosses to *T. aestivum* as male could produce (along with various aneuploids) some fully fertile euploid AABBDD plants. The A and B genomes of those plants were restored from Wichita wheat while their D genome was a mixture of *T. aestivum*, *Ae. tauschii*, and recombinant chromosomes. In field-testing Bikram's backcross lines, Stan noticed that the Wichita phenotype also was recovered very rapidly with backcrossing, not surprising, since two of the three genomes were immediately restored.

With this in mind, Stan set out to use Bikram's method to cross then-recently released hard winter wheat cultivars, such as 'Karl' and 'TAM 107', as well as new breeding lines, with a set of geographically

diverse *Ae. tauschii* accessions and A-genome diploids that John and Bikram had found to have seedling resistance to pests and diseases, most prominently leaf rust. Those crosses led to the development of lines, mostly BC_2-derived, with resistances to leaf rust, soil-borne mosaic virus, spindle-streak mosaic virus, wheat curl mite, Septoria leaf blotch, tan spot, and powdery mildew, the last in cooperation with North Carolina State University and USDA–ARS in Raleigh. Gina Brown-Guedira took over Stan's position in 1997 and continued to work with Bikram on developing germplasm from crosses with both *Ae. tauschii* and *Triticom timopheevii* subsp. *armeniacum*. Forty-nine resistant lines became joint USDA–WGRC germplasm releases between 1985 and 2011, and many more were tested in regional germplasm nurseries and used as parents by breeders.

In the early days of developing germplasm from crosses with progenitor species such as *Ae. tauschii*, Bikram, John, Stan, and others had to make many crosses with aneuploid stocks to locate genes to chromosomes and with other resistant lines for allelism tests to determine whether a novel gene was being transferred. This work became more laborious, time-consuming, and error-prone as the number of D-genome-derived resistance genes grew. So when molecular-marker technology became feasible in wheat, Bikram and Bernd Friebe began using it in the development of new germplasm, streamlining the process considerably. RFLP markers and gliadin proteins also were used to demonstrate the vast reservoir of genetic variation available in *Ae. tauschii* (Lubbers et al. 1991). Compared with the paucity of variation in the D genome of hexaploid wheat (Kam-Morgan et al. 1989), this work provided further rationale for exploiting the wild D genome for wheat improvement.

Bikram realized, however, that some agronomically important traits, such as resistance to wheat streak mosaic virus were not found in species of the primary gene pool of wheat but were present only in the more distantly related species of the tertiary gene pool. Actually, one of Bikram's major responsibilities when he was hired at Kansas State University was to develop wheat germplasm with resistance to wheat streak mosaic virus, which is a serious problem in Western Kansas especially. This work, first initiated by Hari Sharma, produced new hybrids between wheat and several *Agropyron* species (Sharma and Gill 1983). Realizing that gene transfer from these species is more difficult and cannot be achieved by homologous recombination, directed chromosome engineering using the *ph1b* gene was begun.

Bernd Friebe came to the WGRC in early 1989 on a sabbatical to work on standardizing the C-banding nomenclature system for wheat. During this time, C-banding and *in situ* hybridization analyses, both pioneered

for wheat in Bikram's laboratory (see below sections), were well established. In combination, these techniques proved to be a very powerful tool for characterizing wheat-alien germplasm. Chromosome banding or fingerprinting identified the chromosomes involved in translocations and genomic *in situ* hybridization or chromosome painting determined the size of the alien segments. One of the first problems they tackled was using such a molecular cytogenetic approach to characterize a set of wheat streak mosaic virus-resistant wheat–*Thinopyrum intermedium* lines that was produced in the 1970s by Wells and coworkers in South Dakota. Screening of this germplasm by chromosome fingerprinting revealed that most had a complete *Th. intermedium* chromosome, either added or replacing wheat chromsomes 4A and 4D in the derived substitution lines, indicating their homoeology to group-4 chromosomes (Friebe et al. 1991). In one of the lines, however, only the short arm of a *Th. intermedium* chromosome was present and translocated to the long arm of wheat chromosome 4D forming a Robertsonian translocation. This line was genetically compensating, and because of the wheat streak mosaic virus resistance gene, it was agronomically useful. The gene was designated as *Wsm1* and conferred immunity to the virus.

Wsm1 was transferred to Kansas winter wheat, but it turned out that a whole *Th. intermedium* chromosome arm had too many deleterious genes and caused a yield penalty. Using the *ph1b* wheat mutant stock to induce homoeologous recombination between wheat and alien chromosomes, they recovered wheat–*Th. intermedium* recombinants with shortened alien segments that still retained the *Wsm1* resistance gene (for review see Qi et al. 2007). The wheat streak mosaic virus-resistant wheat–*Th. intermedium* recombinant chromosomes were transferred to Kansas winter wheat, should have no yield penalty, and can be used in wheat improvement. As a bonus, it turned out that the *Th. intermedium* segment in these recombinant chromosomes also conferred resistance to the *Triticum* mosaic virus, a new virus disease that recently had emerged in the Great Plains (Friebe et al. 2009). Continuing this work, a second source of wheat streak mosaic virus resistance (*Wsm3*) derived from a different *Th. intermedium* chromosome, but only available as a whole-arm wheat–*Th. intermedium* translocation, was released. This line will need further chromosome engineering before the gene can be used in cultivar improvement (Liu et al. 2011).

F. Establishing the Wheat Karyotype

Giemsa C-banding is still widely used to identify plant chromosomes and distinguishes all 21 chromosome pairs of bread wheat.

In Cambridge, England, during the 7th International Wheat Genetic Symposium, Bikram headed a committee to develop a standard karyotype and nomenclature system to describe the chromosome bands of wheat. Together with Bernd Friebe and Takashi Endo, a standard karyotype of wheat was published in 1991 (Gill et al. 1991). For the first time, Giemsa C-banding chromosome fingerprinting proved to be a fast and very reliable technique to identify wheat chromosomes. Bikram and Bernd developed standard karyotypes for most of the related *Aegilops* species, providing insight into the evolutionary relationships among these species (Friebe and Gill 1996). Together with *in situ* hybridization analysis, another technique refined in Bikram's laboratory, the molecular cytogenetic characterization of wheat-alien translocations conferring resistance to diseases and pests was accomplished (for review see Friebe et al. 1996).

Just how powerful were these new techniques? Bob McIntosh visited the WGRC in 1994. He was interested in mapping the rye-derived, leaf rust resistance gene *Lr45* using monosomic analysis. By 1992, he had analyzed 19 of the 21 cross combinations except those involving chromosomes 2A and 1D, suspecting that, most likely, either chromosome was involved in the *Lr45* transfer. Within a few days, Giemsa C-banding showed that the chromosomes involved in the *Lr45* transfer were wheat chromosome 2A and rye chromosome 2R. *In situ* hybridization revealed that the complete long arm and about half of the short arm of rye chromosome 2R was translocated to the distal half of the short arm of wheat chromosome 2A (McIntosh et al. 1995).

G. Birth of the Chinese Spring Deletion Stocks

Forty years ago, Master's degree student Takashi Endo, working under the guidance of Prof. Koichiro Tsunewaki, noticed a strange phenomenon in the fertility of backcross progeny that retained an *Ae. triuncialis* chromosome and that this chromosome was indispensable to fertile gametes, although he was not sure at that time if the alien chromosome itself had a gametocidal effect on gametes lacking the alien chromosome. After visiting S.S. Maan at North Dakota State University in 1981, Endo moved to Bikram's laboratory at Kansas State for 5 months, the beginning of a long-term collaboration. During this time, Endo was introduced to chromosome banding and continued to improve the techniques after his return to Japan. Thanks to chromosome banding, he found that some chromosomes caused sublethal chromosomal breakage in gametes and that resultant chromosomal structural changes could be established in the subsequent generations.

Around 1985, Sir Otto Frankel, a Fellow of the Australian Academy of Science and Honorary Member of the Japan Academy, was visiting Nara, Japan. Over dinner, Endo told Sir Frankel about the chromosomal aberrations, and he mentioned that he would tell the story to Bikram, who was visiting Australia on sabbatical leave. Bikram took an interest and encouraged Endo to produce wheat stocks carrying deletions. Eventually, Endo received a grant sponsored by the Japan Society for the Promotion of Science to visit Bikram's laboratory during his summer vacation for several years. The homozygous or heterozygous deletions were screened and grown at KSU. Root tips of the deletion heterozygotes were sent to Japan and the data sent back to KSU. Later, Bikram obtained funding from the USDA for RFLP mapping of the deletion lines. The deletion line set was released for public use in 1996 (Endo and Gill 1996). Since then, other similar chromosomes have been isolated in common wheat from different *Aegilops* species, and they were named "gametocidal" chromosomes, which were demonstrated to induce chromosomal breakage in gametes lacking them.

H. FISHing in the Wheat Gene Pool

After his pioneering research work of using C-banding to identify individual wheat and rye chromosomes, Bikram continued to pursue the development of new techniques for reliable identification of wheat chromosomes. Lane Rayburn, from the laboratories of J.D. Smith and Jim Price at Texas A&M University, joined Bikram in 1984 and together they published the first results of nonradioactive DNA *in situ* hybridization (ISH) in plants (Rayburn and Gill 1985). Using a biotin-labeling system to map the repetitive DNA probe, pSc119, signals were visualized using a horseradish peroxidase-based detection system. Although unable to identify all 21 wheat chromosomes, the potential of ISH for chromosome identification was established, because different probe or probe combinations can potentially be used to generate different hybridization patterns on individual chromosomes. Thus, ISH-based chromosome identification systems would be more versatile than the traditional chromosome banding systems. The pSc119 probe has since become one of the most frequently used repetitive DNA probes in plant cytogenetics research.

In the 1980s, fluorescence-based detection systems (FISH) became the choice for visualizing ISH signals. Bikram had a clear vision of the potential of FISH and strongly encouraged and supported several students, postdocs, and visiting scientists to use FISH in their research projects. Several influential, FISH-based research papers were published

in early 1990s, especially using FISH to detect alien chromosomal segments that were transferred into wheat cultivars or breeding lines (Friebe et al. 1993; Mukai et al. 1993; Jiang et al. 1994). As a graduate student, Jiming Jiang worked with Bikram from 1989 to 1994 developing techniques that combined FISH with chromosome banding techniques by sequentially performing the two techniques on the same chromosome preparations, combining the power of the chromosome identification from both (Jiang and Gill 1993, 1994; Jiang et al. 1994).

FISH mapping on plant chromosomes using DNA probes as small as few kilobases was a highly attractive technique to many plant and animal labs in late 1980s and early 1990s. Such techniques would allow physical mapping of genetic markers, such as the popular RFLP markers, directly on chromosomes and to integrate genetic maps with physical maps. Bikram's lab also attempted to develop FISH techniques for mapping small DNA probes, however, only inconsistent results were obtained in the early experiments.

The Gill lab shifted its attention to using large genomic DNA clones, such as yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC) clones, as FISH probes. Initial experiments using maize YAC clones as probes did not produce successful results. But BACs from sorghum and rice were instantly successful (Woo et al. 1994; Jiang et al. 1995). Bikram's lab was the first to utilize BAC clones for FISH mapping in plants, and FISH using BAC clones anchored by genetic markers was to become the most popular methodology for integrating genetic linkage maps with chromosomal maps (Jiang and Gill 2006).

I. Gene-Rich, High-Recombination Regions

Initially, C-banding was the primary technique used for isolating and characterizing the deletion stocks. But because a large number of deletions were isolated for each chromosome (an average 20/chromosome), it was difficult to order the deletion breakpoints based on cytology alone. In the late 1980s, Bikram's first graduate student, Lauren Kam-Morgan had carried out a pioneering study that produced the first DNA restriction fragment length polymorphism (RFLP) based linkage map of a wheat chromosome (Kam-Morgan et al. 1989). Another graduate student, Kulvinder Gill, continued this work and made the first RFLP linkage map of *Ae. tauschii* (Gill et al. 1991). While attending a workshop on RFLP mapping in New Delhi, India, in 1991, Bikram remembers teaching during the day, working on a USDA grant proposal, in long hand, at night, and faxing copies during the day, for over three days! The proposal, "Cytogenetically based physical map of wheat

genome," was rated as the top proposal by the USDA panel and was funded for the full amount of $300,000.

Kulvinder remained at KSU as a postdoc and later, as a senior scientist, and assumed the responsibility for the group-1, -5, and -6 chromosomes (Gill et al. 1993, 1996a,b). While on a trip to Madison, Wisconsin, Bikram attended the thesis defense of Joanna Werner (a student of the late Stan Peloquin) and recruited her to take charge of group-7 chromosomes (Werner et al. 1992). At Kansas State, a postdoctoral student of Scot Hulbert, Donna Delaney, made physical maps of the group-2 and -3 chromosomes (Delaney et al. 1995a,b). Bikram's graduate student Leigh Mickelson-Young made the group-4 chromosome maps (Mickelson-Young et al. 1995). This large mapping effort led not only to the characterization of the deletion stocks but also revealed gene-rich, high-recombination regions most commonly localized to the distal regions of chromosomes (Werner et al. 1992; Gill et al. 1993). The proximal chromosomal regions showed suppression of recombination and were gene poor. Many wheat-specific genes were associated with distal high-recombination regions (See et al. 2006). C-banding had allowed the partition of wheat chromosomes into the biologically meaningful heterochromatic and euchromatic regions, and deletion stocks permitted the targeted mapping of these regions and opened new possibilities for exploring cereal chromosome biology and the positional cloning of many genes crucial to the biology of the wheat plant.

J. The *Q* Gene Story

Justin Faris started as a Ph.D. student in Dr. Gill's laboratory in 1995. After much discussion and lab meetings, they concluded that cloning one or more genes from wheat using map-based approaches was imperative. At that time, no wheat genes had been cloned using map-based methods, and Bikram felt it was important to help demonstrate to the community (and funding agencies) that, despite the large genome and polyploid nature of wheat, map-based cloning in wheat was indeed feasible (other wheat scientists, including Beat Keller and Jorge Dubcovsky, also felt this way and initiated the cloning of wheat genes as well). Justin was charged with leading research to clone the *Q* gene with Li Huang working toward cloning *Lr21*.

The *Q* gene was targeted for cloning in Bikram's lab for two primary reasons. First, Bikram had a long-standing interest in wheat evolution and domestication. The major domestication gene primarily governing the free-threshing character, *Q* also pleiotropically influences many other domestication-related characters (for review see Faris et al. 2005).

Second, Bikram had assembled a large number and variety of genetic and cytogenetic stocks involving the critical chromosome 5A to expedite the work. Two deletion lines differing for a submicroscopic segment of 5A containing the *Q* gene, 5AL-7 and 5AL-23, provided ideal templates for the first step toward cloning, developing markers and saturation mapping of the *Q* locus. Justin compared the two deletion lines side-by-side using RFLP markers, RNA differential display technology, and AFLPs. Fragments present in 5AL-23 but absent in 5AL-7 were cloned, confirmed, and mapped. From this work, Justin developed 18 markers spanning about 20 cM within the deletion interval defined by the breakpoints the two deletion lines. In 1999, Justin graduated and joined the USDA–ARS at Fargo, North Dakota. This work was published soon thereafter (Faris and Gill 2002).

Although the *Q* gene was not yet cloned, the foundation was laid and Bikram allowed and encouraged Justin to continue working on *Q* through close collaboration. A chromosome walk was initiated using the tightly linked markers, a BAC contig spanning the *Q* gene was assembled, and a candidate gene was identified (Faris et al. 2003). The final phase of cloning the *Q* gene involved validation of the candidate gene followed by structural, functional, and phylogenetic analysis. Kristin Simons, who received her Ph.D. with Bikram at KSU, split her time between Bikram's lab at KSU and Fargo, ND, in 2004–2006. She showed that *Q* was an AP2 plant transcription factor, the *Q* and *q* alleles differed for a single amino acid, and also that *Q* alleles are expressed at higher levels than those of *q* (Simons et al. 2006). Kristin also demonstrated the dosage and pleiotropic effects of *Q* in transgenic plants and that the mutation that gave rise to the *Q* allele occurred only once during domestication. These results shed much light on the events that shaped domestication of our modern durum and common wheat cultivars. Collaborative research between the Faris and Gill labs related to *Q* and domestication continues. The team exploited the cloning of *Q* to investigate the structure and function of the *q* homoeoalleles (Zhang et al. 2011).

Bikram's contributions to wheat domestication studies were not limited to the collaboration on *Q* with Justin. Wanlong Li worked in his laboratory on the genetics of the brittle rachis (*Br*) genes (Li and Gill 2006); and Ph.D. student Shilpa Sood conducted genetic studies on the tenacious glume trait governed by the *Tg* and *Sog* genes in wheat and its relatives (Sood et al. 2009). Bikram's contributions to our current knowledge of the genetics of wheat domestication are quite significant and, at the same time, his guidance served as a launching pad for several scientists' careers.