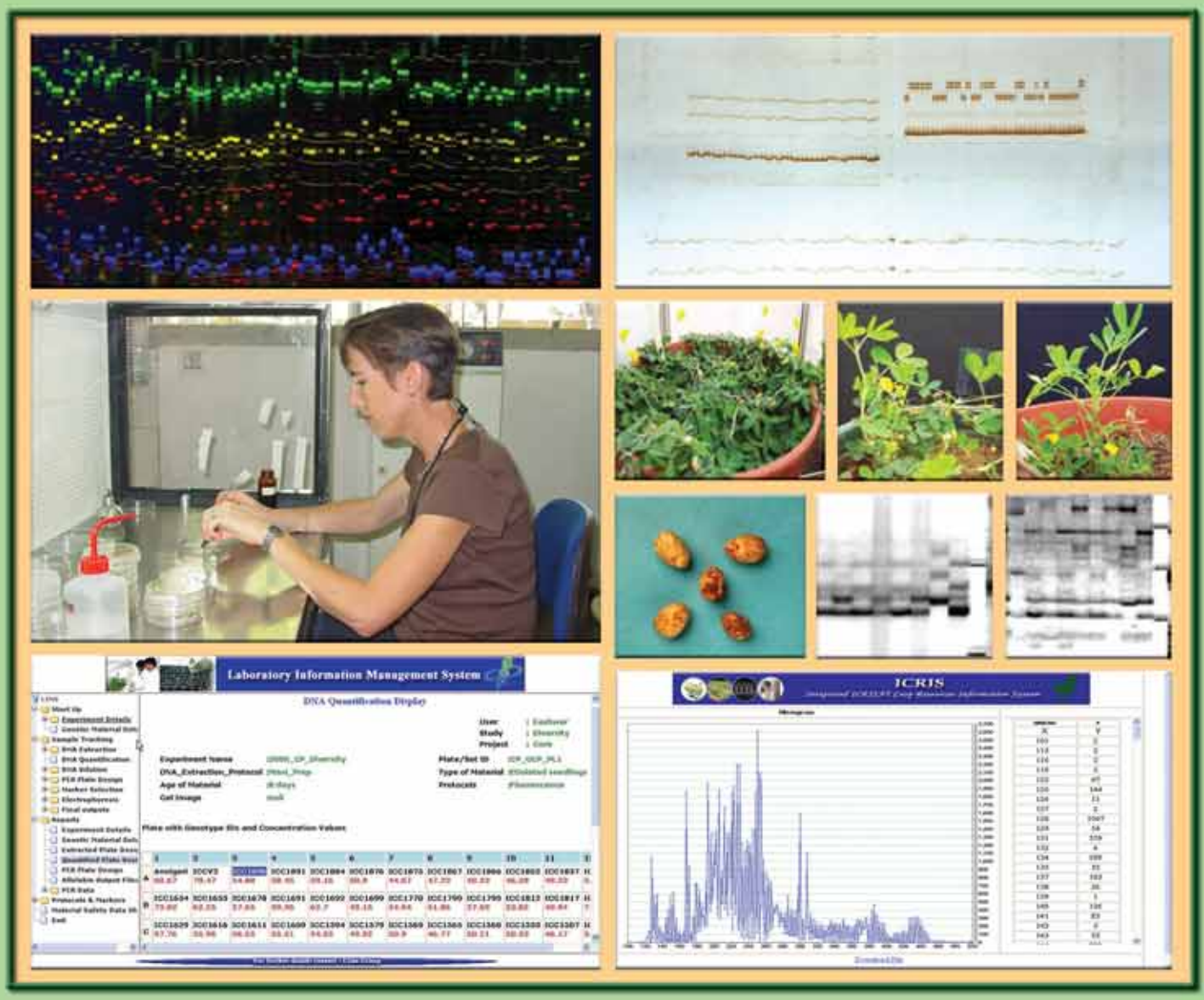
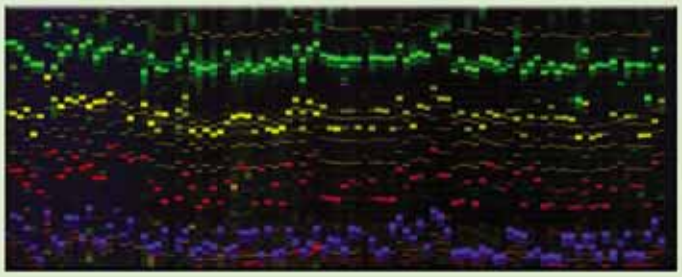
Harnessing Biotechnology for the Poor

Archival Report 2005

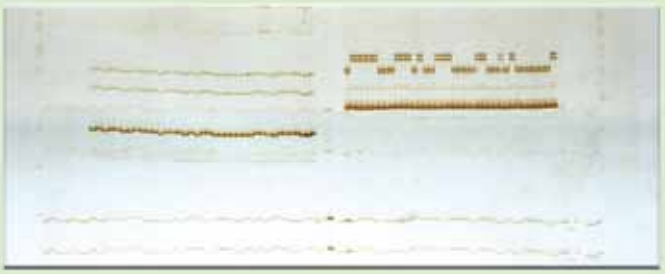


International Crops Research Institute for the Semi-Arid Tropics

Description of Front Cover Photographs



Genotyping of chickpea germplasm collection with 4 microsatellite markers using multiplexing on ABI 3700 sequencer.



SSR genotyping of the groundnut mapping population (TAG 24 x ICGV86031) with polymorphic microsatellite markers using PAGE.

African scientist carrying out genetic transformation studies

Wild species and pollen parent *Arachis kretschmeri*

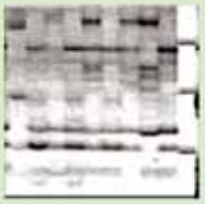
F_1 hybrid between *A. hypogaea* cv ICGS 44 × *A. kretschm*

Backcross (BC_1) from the cross *A. hypogaea* × *A. kretschmeri*

Laboratory Management Information System

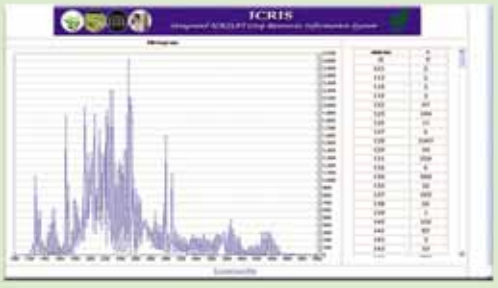
Seeds from BC_1 hybrid from the cross *A. hypogaea* × *A. kretschmeri*

SSR primer 2A06 analysis of F_1 hybrids from the cross *A. hypogaea* × *A. kretschmeri*



RAPD primer OPH 03 analysis of F_1 hybrids from the cross *A. hypogaea* × *A.* kretschmeri

Interspecific hybridization between *Arachis hypogaea* x *A. kretschmeri* (section *Procumbentes*)



Integrated Crop Research Information System

For Further Information
Dr Dave Hoisington
Global Theme Leader
Biotechnology
ICRISAT, Patancheru 502 324
Andhra Pradesh, India

Tel: +91 40 30713071
Fax:+91 40 30713074
E-mail: D.Hoisington@cgiar.org
http://www.icrisat.org/gt-bt/gt-bt.htm

Harnessing Biotechnology for the Poor

[Global Theme 1 – Biotechnology]

Archival Report 2005

International Crops Research Institute for the Semi-Arid Tropics
Patancheru 502 324, Andhra Pradesh, India

Foreword

Scientists in the Global Theme on Harnessing Biotechnology for the Resource Poor continue to achieve remarkable success in applying the tools of modern science in a number of research projects addressing important abiotic, biotic and genetic resource constraints affecting ICRISAT's mandate crops.

The development and application of molecular markers for genetic analysis and marker-assisted breeding continues to be a major emphasis. For species like sorghum, these tools are in abundance and the sequencing of the gene-rich regions of sorghum by the US Department of Energy's Joint Genome Initiative will surely provide the needed resources for our on-going and future applications. For the other ICRISAT species, molecular tools are being developed by a few groups, e.g., for pearl millet, chickpea and groundnut, although for some species like pigeonpea and finger millet, the efforts are extremely limited and will require significant investment by ICRISAT and its partners to provide the necessary tools.

The tools that are available have been applied by the scientists in the fingerprinting of a large collection of germplasm in each of the species. This is now almost complete for sorghum and chickpea, in the final stages for groundnut and pigeonpea, and is beginning for pearl millet and finger millet. These results are providing a better understanding of the genetic diversity available and opening new opportunities for using this diversity in the breeding endeavors. We have also been able to initiate a project on sorghum diversity in Eastern and Southern Africa with the NARS.

Mapping of a range of traits across the species is well underway. For some traits, such as downy mildew resistance in pearl millet, stay green and *Striga* resistance in sorghum, we are using the linked markers in marker-assisted selection programs – with our NARS partners in South Asia and Africa. Of particular importance are the strong efforts to understand the genetics of traits associated with improved drought tolerance. These are likely to have significant impacts in improving our crops for this critical constraint to productivity in many of our crops.

When sufficient heritable diversity is not present within the cultivated species, we are using wide-hybridization and genetic engineering as powerful strategies. Through embryo rescue and tissue culture, we have produced a number of interspecific hybrids in the legumes that have enhanced insect and disease resistance. The production of novel groundnut varieties through crosses of diploids is very promising. All of these are being further backcrossed to cultivated varieties and molecular markers linked to the introgressed segments so that these can receive broader applications in the breeding programs of ICRISAT and our partners.

Through the insertion of specific genes for enhanced disease and pest resistance, drought tolerance and enhanced nutritional quality, we have available numerous transgenic events that are being evaluated for the targeted phenotype. Many of these have reached the field testing stage and are being considered for further evaluation with our NARS partners in India and Africa. Our optimism for the technology has led us to initiate stronger activities in Africa through staff appointments and research in Kenya and South Africa.

We continued to strengthen and widen our public, private and NARS partners for successfully implementing biotechnology research strategies and collaborative research. We believe the future looks very promising for providing more efficient and effective tools for improving ICRISAT's crops and for our part in ICRISAT's mission to improve the food and nutritional security and livelihoods of the resource poor in the semi-arid tropics.

Dr Dave Hoisington
Global Theme Leader
Harnessing Biotechnology for the Poor

Contents

Section 1

Overview of ICRISAT Global Theme: Harnessing Biotechnology for the Poor

David Hoisington

Global Theme Leader

Scientists in the Global Theme continue to achieve remarkable success in a number of projects addressing abiotic, biotic and genetic resource constraints to ICRISAT's mandated crops.

Goal

The goals of this theme are to harness the power of biotechnology to augment the gains achievable through plant breeding and to provide cost-effective diagnostics for critical plant diseases and associated toxins.

Objectives

In its efforts to achieve the overall goal, the Theme focuses on the following two major objectives:

1. To improve the efficiency, effectiveness, speed and precision of plant breeding for abiotic stress tolerance, pest and disease resistance, better agronomic traits, and improved food, feed and fodder quality; and
2. To develop diagnostic tools for the detection of viral infections, toxic contaminants of crops and crop-based products, presence of transgenes, and purity of seed production systems.

Introduction

The Theme is organized into the following three main Global Projects:

Global Project 1: Improved abiotic stress tolerance, agronomic and quality traits via the application of genomics, genetic engineering and wide-hybridization;
Global Project 2: Enhanced resistance to insect pests and diseases via the application of genomics, genetic engineering, wide-hybridization and diagnostics; and
Global Project 3: Better understanding and use of agro-biodiversity through the application of genomics and bioinformatics.

Each of these projects depends on the proper application of a range of technologies available within ICRISAT and/or our global partners. Activities within each Global Project are conducted where most effective and efficient – in ICRISAT's laboratories in Asia and Africa, and/or our many partner institutes around the world.

Projects in the Global Theme continue to tap the potential of biotechnology tools for enhancing the speed, precision, efficiency and value addition to many aspects of plant breeding including addressing complex traits that have remained intransigent to conventional breeding. We recognize that biotechnology has enhanced the efficiency, effectiveness, speed and precision of plant breeding; however, breeding for crop productivity in marginal areas requires a complex multidisciplinary collaboration to facilitate the development of effective solutions. Establishing and backstopping such networks is a primary focus for ICRISAT in Africa and Asia. On this basis, a high-throughput DNA

marker laboratory has been established at ICRISAT-Patancheru to strengthen our capacity in molecular breeding with particular reference to liberating the value encapsulated in our germplasm collections. Major advances have already been made in the molecular breeding of sorghum and pearl millet and in the development of tools for mapping important traits in chickpea, groundnut and pigeonpea.

We firmly believe that with our "partnership-in-progress" there are good chances for all of us to succeed. The establishment at ICRISAT-Patancheru of the Agri-Science Park and an Agri-Business Incubator strengthen strategic alliances with the private sector for partnerships to facilitate research-for-development and multi-sector collaboration in biotechnology. This leads to a broader scope for applying science expertise and tools to any crop of importance in the Semi-Arid Tropics.

GT-Biotechnology is actively involved in all three Challenge Programs. In the HarvestPlus Challenge Program, we are working on improving the pro-vitamin A content of groundnut and pigeonpea by increasing the β–carotene level using genetic engineering, enhancing the nutritional quality of pigeonpea for sulfur-rich amino acids using genetic engineering, and improving the micronutrient content in sorghum using genomics. Under the Generation Challenge Program, ICRISAT projects include the assessment of molecular diversity in sorghum, pearl millet, chickpea, pigeonpea and groundnut; marker-assisted improvement of drought tolerance in sorghum, pearl millet and chickpea; genetic engineering of drought tolerance in groundnut; and the development of various bioinformatics tools. In the Water and Food Challenge Program, we are using molecular markers to backcross the stay-green trait into early maturing sorghum varieties for West Africa.

Global Project 1: Improved abiotic stress tolerance, agronomic and quality traits via the application of genomics, genetic engineering and wide-hybridization

Drought is globally the most important constraint to crop productivity and with predictions of greater water scarcity in the future, drought is likely to remain the number one constraint. As options for irrigation are often not available in the semi-arid tropics (SAT), it is critical that genetic enhancement strategies focus on maximizing extraction of available soil moisture and improving the efficiency of water use in crop establishment, growth, biomass production and seed yield. Genetic improvement for drought tolerance has always been a challenge to conventional breeding approaches that rely on selection for yield in drought-stressed environments. The large genotype × environment interaction for yield in natural stress environments often makes direct selection for yield ineffective. Biotechnological tools provide targeted approaches for improving component traits of drought tolerance, which should be more effective than the conventional breeding methods in developing drought tolerant germplasm.

This global project focuses on identifying molecular markers for the quantitative trait loci (QTLs) controlling traits contributing to drought tolerance/avoidance in pearl millet, sorghum, chickpea and groundnut, and on the marker-assisted introgression of these QTLs into adapted cultivars/farmer parental varieties, elite breeding lines. Based on available information on their relative importance in conferring drought tolerance/avoidance, we have selected traits for improvement in each crop, for example, stay green in sorghum, deep and vigorous roots in chickpea, and water use efficiency in groundnut. In pearl millet, we have pursued QTLs associated with maintenance of grain yield under terminal drought situations.

The global project is also evaluating transgenic technology for developing drought tolerant plants in chickpea and groundnut. The genes presently being tested include *DREB1A* and *P5CSF129A*. The transcription factor, *DREB1A* driven by a drought-responsive *rd29A* promoter is expected to enhance tolerance to several abiotic stresses, such as drought, chilling temperature and salinity. The gene *P5CSF129A* increases proline accumulation and improves tolerance to osmotic stress.

Another important abiotic stress being targeted is soil salinity, which is commonly found in arid regions that rely on irrigation for agriculture. We are pursuing efforts to identify sources of salinity tolerance in all five ICRISAT mandate crops. Existing mapping populations have been developed in pearl millet that will be used for identification of QTLs for salinity tolerance. In sorghum, new mapping populations are being developed for this purpose. Similar work will be initiated in ICRISAT mandate legume crops when suitable parents are identified for development of mapping populations.

We are also seeking to improve the ability to obtain and utilize key soil nutrients in our mandate crops. For example, we have found significant variation in phosphorous (P) acquisition ability from low P soils in pearl millet. We are investigating the underlying mechanisms in P acquisition ability of plants and making efforts to identify markers for this important trait.

In the past, the breeding efforts in crop improvement have largely focused on genetic enhancement of yield potential and resistance to biotic and abiotic stresses. The emphasis on quality traits is increasing in recent years and is expected to increase in the coming years. We are presently targeting the improvement of the pro-vitamin A (β-carotene) content in pearl millet, groundnut and pigeonpea; methionine content in pigeonpea; stover ruminant quality in pearl millet; and feed and fodder quality in groundnut and pigeonpea. Marker-assisted breeding will be used for improvement of all these traits, except for β-carotene content in groundnut and pigeonpea, and methionine content in pigeonpea, for which transgenic technology is being used.

The concerted and focused efforts underway will lead to development of diversified breeding populations/lines of ICRISAT mandate crops with improved tolerance to abiotic stresses, improved nutrient uptake and utilization, improved nutritional quality of grain, and improved quality of feed, fodder and stover. We will make these available to all public and private sector plant breeders and seed producers globally, who will, in turn, use these to produce locally adapted varieties with improved tolerances and quality traits for SAT farmers who depend upon our crops for their livelihoods.

Highlights for 2005

Marker-assisted introgression of drought tolerance QTLs in pearl millet

- Experiments were conducted to test whether roots have any role to play in the QTLs identified for terminal drought tolerance in pearl millet. The roots of tolerant genotypes reached a length of up to 240 cm in those plants exposed to water stress, whereas no sensitive pearl millet genotype had roots longer than 210 cm under water stress. The pearl millet genotypes having tolerance to terminal drought also had larger "investments" in deeper rooting than the drought sensitive pearl millet genotypes. Expressed as a percentage of roots in the 0-30 cm layer (to compare to shallow rooting), tolerant pearl millet genotypes had 15-20% of the roots present in soil layers deeper than 150 cm, whereas sensitive pearl millet had less than 5% of roots deeper than 150 cm.
- Products of marker assisted backcrossing (MABC) were advanced by one generation for development of drought tolerance QTL-NILs in the background of elite maintainer line 841B

having introgressed alleles from LG 1 and LG 6 of donor 863B. Products of MABC for drought tolerance QTL-NILs with LG 2 substitutions from donor 863B in the background of elite maintainer line 841B were tested in second year. Conducted fourth year of testing of products of MABC for drought tolerance QTL-NILs with LG 2 substitutions from donor PRLT 2/89-33 in the background of elite restorer line H 77/833-2. Products of MABC for drought tolerance QTL from LG 2 of donor PRLT 2/89-33 in background of elite pollinator H 77/833-2 were retested.

Marker-assisted breeding for stay-green QTLs in sorghum

- Experiments were conducted to test whether roots have any role to play in the QTLs earlier identified for terminal drought tolerance in sorghum (QTLs that were detected for the stay-green component of terminal drought tolerance). Roots of stay-green trait donors and stay-green QTL introgressed materials reached a length of at least 270 cm under water stress, whereas senescent genotypes ISIAP Dorado and R 16 had a maximum root length of 210 cm. Again, expressed as percentages of roots in the 0-30 cm layer stay-green sorghums had 15-35% of the roots present in soil layers deeper than 150 cm, whereas senescent sorghums had only 5-10% of roots deeper than 150 cm.
- Progenies from MABC introgression of several putative stay-green QTL into released cultivars ISIAP Dorado were evaluated. Genetic differences in stay green expression were clear in the case of the highly senescent R 16, but less so in the case of the moderately senescent ISIAP Dorado. None of the backcross progenies expressed the stay-green trait to the same degree as the donor parent B35, but most have only one or two stay-green QTL, in contrast to B35, which has a larger number (six or more).
- BC_3F_2 and BC_4F_2 plants homozygous for donor parent alleles at SSR marker loci flanking each of six individual stay-green loci (i.e., single-QTL introgression lines for stay-green QTLs stgA, stgB, stg1, stg2, stg3 and stg4) from donor parent B35 in the genetic background of ICSV 111 (released in Ghana as 'Kapaala') and its sub-selection S 35, were identified and their selfed seed harvested. These included ten BC_4F_2 plants expected to be homozygous for stgA, three BC_4F_2 plants expected to be homozygous for stgB, six BC_3F_2 plants expected to be homozygous for stg1, ten BC_3F_2 plants expected to be homozygous for stg2, two BC_3F_2 and seven BC_4F_2 plants expected to be homozygous for stg3, and four BC_4F_2 plants expected to be homozygous for stg4. The corresponding BC_4F_3 and BC_3F_3 families, along with seed samples of their recurrent and donor parents, were sent to the national sorghum breeding program in Ghana (SARI) for agronomic and farmer-participatory evaluation.

Marker-assisted breeding for drought-avoidance root traits in chickpea

- Two experiments were conducted to assess how much and at what stage deep rooting actually contribute to water extraction, in particular for conferring tolerance to terminal drought. Three genotypes with deep and profuse rooting (ICC4958, Annigeri and ICC8261), and two genotypes with shallow rooting (ICC1882 and ICC283) were used. Preliminary results show that deep rooted genotypes extract more water from the 60-90 cm layer at 25 days after sowing and the 90-120 cm layer at 34 DAS, than shallow rooted genotypes.
- The genetic components of root characteristics were investigated through generation mean analysis, using six basic populations (P1, P2, F_1, F_2, BC_1, and BC_2) of two crosses (ICC283 × ICC8261 and ICC4958 × ICC1882). The additive gene effect played an important role in controlling root length density (RLD) in both the crosses.

- A RIL mapping population developed from the cross ICC4958 (large roots) × ICC1882 (small roots) was evaluated for root traits. The plants were grown in cylinder culture systems and the root traits were studied at 35 DAS. The root system of each entry was divided into 4 pieces of different soil depths (0-30 cm, 30-60 cm, 60-90 cm; and 90-120 cm) and scanned to obtain digital images. Later, the dry weight was recorded. All digital images were analyzed using the image analysis software WinRhizo.

Marker assisted breeding for water use efficiency in groundnut

- The two parents TAG24 (low TE) and ICGV86031 (high TE) were tested along with 318 RIL F8 progenies from their cross. Preliminary analysis indicated a good correlation with data gathered in 2004. This was so despite the fact that the range of TE values obtained in 2005 (1.9 – 2.4) was less than the range obtained in 2004 (2.5 – 3.7). In 2005, we also received the delta $\Delta^{13}C$ data from the 2004 phenotyping experiment and found the well-reported negative correlation between TE and $\Delta^{13}C$.
- Parents (TAG24 and ICGV86031) of the RIL population phenotyped for WUE were screened with 463 SSR markers. Thirty-five markers were found to be polymorphic and were screened on 318 RILs.
- Eighteen groundnut accessions with 7 control cultivars were evaluated for pod yield potential and other traits related to drought. ICGs 5745, 6766, 7243, and 14523, ICG 14475 had 129-150 and 132-153 Specific Leaf Area (SLA) at 60 and 80 days after sowing (DAS) and 42-44 SPAD Chlorophyll Meter Reading (SCMR) at both DAS. ICG 2773 and ICG 5827 produced greater pod yield than the high yielding control cultivars ICG 44 and ICG 76. SCMR and SLA data were recorded groundnut for composite collection consisting of 850 accessions at 60 and 80 DAS. ICGs 2741, 5725, 5728, 6323, and 7878 were identified for high SCMR (53.9 – 61.0).

Transgenic drought tolerance in chickpea and groundnut

- Seven events of the C235 chickpea genotype transformed with *P5CSF* gene were tested in our common dry-down protocol. Higher cumulative transpiration values were recorded in all the transgenics than in C235 wild type under water stress. The total transpirable soil water (TTSW), i.e., the amount of water that plants were able to extract from the soil, was higher in all transgenics than in the wild type under water deficit. Although we found significant differences in TE, we found no advantage of the transgene for TE compare to the wild type in any of the 7 events tested.
- Five transgenic groundnut lines from parent JL24, containing a *DREB1A* gene, plus JL24 were tested for the response of transpiration to progressive soil drying. Results are very promising for several reasons: (i) the transgenic lines RD2 shows a significantly lower FTSW threshold where transpiration declines than JL24; (ii) The transpiration efficiency of this line is circa 50% higher than JL24; (iii) The TE of most transgenic lines is higher under well-watered conditions and it seems to correlate with different stomatal behavior, i.e. stomatal conductance (Gs) was lower in transgenics, in full agreement with the theory explaining differences in TE; (iv) There was a close correlation (R^2=0.87) between the FTSW threshold where transpiration begins to decline and TE, i.e. genotypes with more conservative behavior and early stomatal closure, like JL24, had lower TE than lines having late stomatal closure, like transgenic line 2; and (v) There was no correlation between TE and delta $\Delta^{13}C$.

Mapping salinity tolerance in pearl millet and sorghum

- The 24 parental lines of 12 existing ICRISAT pearl millet mapping populations were further assessed under salinity and three contrasting pairs of parents were identified. These mapping populations are being used to identify QTLs associated with salinity tolerance. Forty TRAP markers based on gene sequences for three enzymes expected to be involved in salinity stress tolerance were developed and mapped.
- SSR-marker-based genetic diversity assessment was initiated for of a set of 30 sorghum genotypes identified as either sensitive or tolerant to salinity stress. Initial results, based on information generated using 76 SSR marker primer pairs, indicate that there is substantial genetic diversity within the range of productive and apparently salinity tolerant breeding lines and elite varieties tested, and that sufficient marker polymorphism is available to allow genotyping of mapping populations that could be produced by crossing selected pairs of salinity sensitive and tolerant genotypes.

Mapping salinity tolerance in chickpea, groundnut and pigeonpea

- A large screening of chickpea genotypes was screened for salinity tolerance. A 6-fold range of variation in the seed yield per pot was observed. Some genotypes had a 20% higher seed yield under salinity than the best released variety for salinity tolerance, CSG8962. The parents of ICCV 2 × JG 62 mapping population showed high contrast for seed yield under salinity. Thus, this mapping population will be used to map QTLs for salinity tolerance.
- A total of 288 groundnut genotypes were tested for salinity tolerance. A 5-fold variation in pod numbers across genotypes was found. Breeding line ICCV92206 was among the best. A poor relation between pod number and seed yield was observed.
- In pigeonpea, a set of genotypes, including newly set up mini-core collection, wild relatives (*acutifolius*, *platycarpus*, *sericeus*, *albicans*), wild derivative from the breeding program, and few hybrids were screened for salinity tolerance. Wide variation for biomass production was found at vegetative stage (biomass at 50 DAS), with a large contrast between accessions C. *scaraboides* and of C. *sericeus*. This opens the possibility to develop mapping populations from interspecific crosses.

Marker-assisted breeding for phosphorus acquisition ability in pearl millet

- A protocol was developed to measure root acidification of pearl millet seedlings in the lab. This protocol combines pH measurement and image analysis and could potentially be used for large screening. The current orientation of this work is to modify this method to make measurement of acidification in older plants and correlate results with measurements at the seedling stage.
- The phenotyping of the testcross hybrids obtained from the combination of four different male-sterile lines and the mapping progenies of the crosses between 81B-P6 and ICMP 451-P8, and between LGD 1-B-10 and ICMP 85410-P7 was undertaken. Plants receiving the low P treatment appeared to be very stunted compared to those receiving the control treatment.
- A series of experiments were conducted to address four issues (i) the differences in Olsen P in the different batches of soil, (ii) the control of plant to plant variation; (iii) the source of nitrogen; and(iv) the possibility to favor initial plant establishment with early application of tiny doses of soluble P.

Genetic engineering of groundnut for enhancement of β-carotene and of pigeonpea for enhancement of methionine and β-carotene

- *Agrobacterium*-mediated genetic transformation of the selected genotypes of groundnut was carried out by using newly constructed binary vectors containing β–carotene genes (*psy1* and *crtB*). The putative transgenic groundnut shoots obtained under antibiotic selection pressure were elongated for rooting followed by their transfer to the containment greenhouse.
- The primary putative transformed pigeonpea shoots obtained under antibiotic selection pressure are being analyzed by PCR analysis for the presence of *SSA* and *psy1* genes. The expression of the *SSA* gene in transgenic tobacco (used as a modal system) was verified by RT-PCR analysis of the T1 seeds.

Improved pearl millet stover quality

- QTL analysis was performed by composite interval mapping for stover quality traits of individual stover fractions (stem, sheath and leaf blade) as well as whole stover samples. For stem fraction digestibility and metabolizable energy content, a major QTL was identified on LG 2, at a position similar to that for drought tolerance alleles from the 863B parent (suggesting it should be simple to combine improved stem digestibility with greater drought tolerance in the genetic background of elite seed parent maintainer line ICMB 841). Another major stover quality QTL was mapped to LG 5, which controlled significant proportions of phenotypic variation for *in vitro* true digestibility and for metabolizable energy content.
- For the putative LG 7 stover quality QTL, RFLP and SSR marker data were used to identify introgression homozygotes for the associated region from donor parent 863B in the genetic background of ICMB 841. Several such plants were identified and their selfed seed harvested for future testcross hybrid production.
- We completed the second and final cycle of FS progeny selection for improved stover quality in the released pearl millet variety ICMV 221. As in the first cycle, there were highly significant differences for all stover quality traits and agronomic variables measured, among the 280 FS progeny evaluated. Selected progenies will be recombined to form both grain and dual-purpose versions of the original variety with improved stover nutritional quality

Global Project 2: Enhanced resistance to insect pests and diseases via the application of genomics, genetic engineering, wide-hybridization and diagnostics

Insect pests, diseases, and the parasitic weed, *Striga* are serious constraints to increase production, productivity, and utilization of sorghum, pearl millet, chickpea, pigeonpea, and groundnut in the SAT. Crop losses due to these pests have been estimated at over US$ 7.4 billion annually. While *Helicoverpa* control is heavily based on insecticides, chemical control of shoot and panicle feeding insects on cereals is beyond the reach of resource poor farmers in the SAT regions in Asia, Africa, and Latin America. For many diseases and *Striga*, cost effective technologies are yet to be worked out. Current sensitivities about environmental pollution, human health and pest resurgence are a consequence of improper use of synthetic pesticides. Host-plant resistance, natural plant products, bio-pesticides, natural enemies and agronomic practices offer a potentially viable option for integrated pest management (IPM). They are relatively safe for the non-target organisms and human beings. Use of modern biotechnological tools such as marker assisted selection, genetic transformation and wide hybridization for developing crop

cultivars with resistance to insect pests and diseases will have a great bearing on future pest management programs. Insect and disease modeling, decision support systems, and remote sensing would contribute to scaling up and dissemination of the IPM technologies. Current research projects in biotechnology, crop improvement and natural resource management focus on the major pests such as pod borers (*Helicoverpa, Maruca*, and *Melanagromyza*), Fusarium wilt and sterility mosaic in pigeonpea; *Helicoverpa*, Wilt, and Botrytis gray mold in chickpea; rossette virus, foliar diseases, aflatoxins and leaf miner in groundnut; *Striga*, grain molds, shoot fly, stem borers, midge, and head bugs in sorghum; and downy mildew, stem borer, and head miner in pearl millet. IPM promotion and capacity building are significant components of research at ICRISAT. The outputs from this research will lead to sustainable management of insect pests and diseases of cereals and legumes based cropping systems, thereby improving the livelihoods of poor people in SAT.

Highlights for 2005

The mapping stem borer/midge resistance in sorghum

- Mapping population based on 296B × IS 18551 (270 lines) was evaluated for resistance to spotted stem borer, *Chilo partellus*, under artificial infestation. Leaf damage rating (DR) varied from 4.0 – 8.0 (1 = <10% leaf area damaged, and 9 = >80% leaf area damaged) and deadheart formation ranged from 5.6 to 90.3% in the mapping population.
- The mapping population, ICSV 745 × PB 15881-3, was evaluated for resistance to sorghum midge, *Stenodiplosis sorghicola*. Midge damage in the mapping population ranged from 2.0 to 9.0 (on a 1 to 9 rating scale, where 1= <10% spikelets with midge damage and 9 = >80% spikelets with midge damage) as compared to 1.0 in the resistance check, ICSV 197, and 7.7 in the susceptible check. Putative QTLs associated with resistance to these insects have been identified.

Mapping shoot-fly resistance in sorghum

- A linkage map based on 296B × IS 18551 has been developed that covers 2165 cM. Composite interval mapping revealed the presence of putative QTLs for all important shoot fly resistance traits, accounting for 6 to 36% of observed phenotypic variances for these traits.
- Three of the four target QTLs from the donor parent IS 18551 were introgressed to the BC_4F_2 generation in the genetic background of shoot fly susceptible elite sorghum hybrid seed parental line, 296B; and all four QTLs were advanced to the BC_4F_2 in the genetic background of shoot fly susceptible elite sorghum hybrid seed parental linem BTx623.

Mapping *Striga* resistance in sorghum

- The non-redundant sorghum EST sequences with SSR motifs were searched against the rice genome sequence in the *Gramene* database (using BLAST). A subset of 600 sorghum EST-SSR loci distributed across regions of the sorghum genome that are syntenic with each of the 12 rice chromosomes (50 each) was selected. A subset of the E36-1 × N 13-based RIL population was used to start mapping some of the polymorphic EST-SSR markers.
- NARS in the Kenya, Mali, Eritrea and Sudan are being assisted to strengthen *Striga* resistance of farmer-preferred sorghum varieties (FPSVs) through a combination of marker-assisted backcrossing (MAB) and farmer-participatory selection. The stability of inheritance of the transferred *Striga* resistance alleles in the FPSVs, the actual out-crossing rates in selected FPSVs, and pollen flow of these FPSVs is being analyzed in order to develop recommendations for variety maintenance and on-farm seed production.

Mapping downy mildew resistance in pearl millet

- The year 2005 saw the first official release for seed multiplication and cultivation of a product of marker-assisted selection, "HHB 67 Improved", for downy mildew resistance in pearl millet. This pearl millet hybrid was identified for release Haryana, India and was later identified (in July) at the national level for cultivation in the arid zone of northwestern India, where extra-early maturing pearl millet hybrid "HHB 67" is been cultivated on nearly 500,000 ha annually.
- QTL analysis of the mapping population progeny derived from cross 841B-P3 × 863B-P2 detected a major QTL on linkage group 4 accounting for nearly 60% of the observed variation in downy mildew incidence. Two resistance QTLs of small effect were also detected (one each on LG 1 and LG 7)

Mapping *Helicoverpa* resistance in chickpea

- Two RIL mapping populations from the interspecific crosses of C. *arietinum* × C. *reticulatum* (ICC 3137 × IG 72953 and ICC3137 × IG 72953) for mapping of QTLs for resistance to the pod borer, *Helicoverpa armigera* were advanced to F_4. Crosses were also made between ICCC 37 and ICC 506EB for initiating development of an intraspecific mapping population for mapping resistance to *H. armigera*.

Mapping *Ascochyta* blight and *Fusarium* wilt resistance in chickpea

- A linkage map of chickpea with 84 markers (82 SSRs and 2 ESTs) was constructed using one F_2 population of an intraspecific cross of ICCV 04516 (AB resistant) and Pb 7 (AB susceptible). The map length was 724 cM with an average density of 8.62 cM. Three AB resistance QTLs were mapped, 1 on LG 3, and 2 and 3 on LG 4. The QTLs identified on LG 4 were validated in another population.
- A set of 84 RILs derived from the cross C 104 ×WR 315 was genotyped using 73 SSR markers and screened for resistance to Fusarium wilt (FW) race-1. Forty-five markers were assigned to nine linkage groups. The gene for FW race 1 was mapped on linkage group 2. Three SSR markers, TA37, TA 200, and TR2 were closely linked to the resistance gene. The former was located at a distance of 0.4 cM, while the latter two markers were located at a distance of 3.5 cM.

Mechanisms, diversity, stability and inheritance of resistance to *Helicoverpa* in wild relatives of chickpea and pigeonpea

- In pigeonpea, Larval Weights of *Helicoverpa armigera* larvae were <20 mg when reared on diets having pod powder of C. *acutifolius*, C. *sericeus* (ICPW 160*)*, C. *scarabaeoides* (except ICPW 137, ICPW 141, and ICPW 152, *P. scariosa*, C. *platycarpus*, and *R. aurea)* compared to 53.3 mg on ICPL 87 and 44.0 mg on ICPL 332. Larvae took >25 days to complete the development when reared on the artificial diet impregnated with lyophilized pod powder of C. *acutifolius* (ICPW 2), C. *lineatus* (ICPW 41), C. *sericeus*, C. *scarabaeoides* (except those reared on ICPW 125), *P. scariosa* (ICPW 207), *R. aurea* (ICPW 210), *D. ferruginea*, and C. *platycarpus* (ICPW 68) as compared to 15.7 days on ICPL 87, 23.3 days on ICPL 332, and 12.7 days on artificial control diet.

Introgression of genes conferring resistance to *Helicoverpa* from wild relatives of pigeonpea and chickpea

- Advanced generation diploid (F_1BC_4) and tetraploid (F_4-F_5) derivatives from the cross, C. *platycarpus* × C. *Cajan*, were screened for resistance to *H. armigera* under unprotected field

conditions. Damage due to *H. armigera* in the wild parent, C. *platycarpus*, was <1%. Damage in cultivated parent, ICPL 87 was 80%. Damage in diploid derivatives was nearly 20% and in tetraploid derivatives 8%. Nearly 25% pod damage was recorded in the second and third flush in the diploid hybrids.

- In chickpea six lines from the cross ICCV 92318 × IG 72934 suffered nearly 20% pod damage, while one line showed <10% pod damage.

Wide hybridization for resistance to Botrytis gray mold and Ascochyta blight in chickpea

- Over 100 wild C*icer* accessions were evaluated for résistance to BGM. Three accessions of C. bijugum, 21 accessions of C. *judaicum* and one accession of C. *reticulatum* were found to be resistant to BGM with a mean disease score of < 2.5 (on a 1 to 9 rating scale) as compared to 9.0 in the susceptible check, JG 62
- Wild C*icer* accessions IG 17159 and IG 73074, which have moderate level of resistance to *Botrytis* gray mold were used in the crossing program. Screening data suggested that the BGM in wild C*icer* accessions, IG 17159 and IG 73074, was monogenic and recessive.

Wide hybridization for resistance to early/late leaf spots and Aflatoxins in groundnut

- A total of 105 lines of advanced generation interspecific derivatives involving *A. cardenasii*, *A. stenosperma*, *A. kempf-mercadoi* and *A. diogoi* were screened against late leaf spot (LLS) under field conditions. Nineteen lines from *A. hypogaea* × *A. cardenasii*, and one line from *A. hypogaea* × *A. dura* × *A. hypogaea* showed high levels of resistance to LLS (score of 2 (a 1 to 9 scale).
- To generate interspecific amphidiploids, wild *Arachis* from different sections were crossed with *A. hypogaea*. F_1 hybrids were obtained between *A. hypogaea* and *A. diogoi* (section Arachis), BC_1 and BC_2 hybrids were obtained between *A. hypogaea* × *A. chiquitana* (section *Procumbentes*), and two BC_2 hybrid were obtained between A. *hypogaea* × *A. kretschmeri* (section *Procumbentes*).

Transgenic resistance to *Helicoverpa* in pigeonpea and chickpea

- Transgenic pigeonpea plants carrying the *cry1Ac* gene were evaluated during 2004/05 cropping seasons under field conditions. The leaves of three transgenic events reduced the larval weight by 40% after 4 days of infestation as compared to that on the control plants. Pod bioassay of two transgenic events with third-instar *H. armigera* indicated weight gain of 156 to 263% as compared to 357 to 461% in control plants.
- Over 20 transgenic events of chickpea with *cry1Ab* and *cry1A*c genes were bio-assayed for resistance to *H. armigera*. There was approximately 35 to 40% reduction in *H. armigera* larval weight on transgenic chickpea plants as compared to that control plants.

Transgenic groundnut resistance to fungal pathogens and viruses

- For the Indian peanut clump virus (IPCV), contained field trial of the selected 5 events of groundnut transgenic plants having coat protein or replicase genes was carried out at ICRISAT, Patancheru. Two transgenic plants showed initial infection (tested virus positive in ELISA), but in subsequent assays were virus negative.

- For the peanut bud necrosis virus (PBNV), the transgenic groundnut (cv. JL 24) plants of 48 independent events were produced with two vector constructs encoding nucleocapsid protein (NP) gene of PBNV through two different transformation systems. Thirty-five independent events were evaluated for resistance to PBNV by using 100-times dilution of the virus inoculum. Of these, 24 lines showed less incidence (30%) compared to untransformed controls (100%).
- Transgenic groundnut containing rice chitinase gene was evaluated for *A. flavus* resistance by *in vitro* seed inoculation. Seed infection rate in these varied from 0 to 100%. Twenty-two plants that had 0 to ≤10% seed colonization were selected and advanced to T_4 generation, which are under testing.

Diagnostic tools for mycotoxins and viruses

- Exposure of humans and animals to Aflatoxin (AFB1) results from the consumption of contaminated food. AFB1 covalently binds to lysine moiety of serum albumin (AFB1-lys adduct). AFB1-lys adduct has been identified as a useful biomarker to determine the human exposure to aflatoxins, and to assess the exposure risk among various sections of population. An indirect ELISA has been developed for quantitative estimation of AFB1-lys adducts in human serum albumin. ELISA test was validated using reference antibodies by analyzing Hepatitis B virus positive samples.

Characterization of pigeonpea sterility mosaic virus (PPSMV) isolates and development of diagnostic tools

- PPSMV isolates can broadly be grouped as B and P types. The PPSMV isolates within each group have distinct physico-chemical characteristics. The B type isolates can overcome host-plant resistance selected against P types. The B types occur in northern and southern regions, and P types occur in the central regions of India.

Non-target effects of transgenic crops on beneficial natural enemies of crop pests

- There were no adverse effects of Bt toxins on the fecundity of the *H. armigera* larval parasitoid Campoletis *chloride*. The adverse effects of Bt toxins on C. *chloride* were through early mortality of *H. armigera* larvae, and not through direct effects of the Bt protein no traces of Bt toxin protein were found in the C. *chloride* cocoons and adults with the ELISA test.

Global Project 3: Better understanding and use of agro-biodiversity through the application of genomics and bioinformatics

Plant breeding efforts aimed towards increasing food security require genetic resources as a critical component - both for short-term gains and long-term increase in productivity. The International Agricultural Research Centers have responded to the threat of genetic erosion in economically useful plant species by developing a global network of genebanks for *ex situ* conservation of genetic diversity. Chapter 14G of Agenda 21 of the United Nations Conference on Environment and Development (UNCED) and the Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture (GPA) endorsed by the Conference of the Parties to the Convention on Biological Diversity (CBD) underscore the importance of and the responsibilities on the large *ex situ* collections held by the CGIAR Centers, including ICRISAT.

ICRISAT assembled a large collection of germplasm of its mandate crops from threatened areas of biodiversity. The assembled germplasm needs to be maintained using appropriate procedures and through establishment of safe, efficient and cost-effective management systems. While ICRISAT collections represent 70-80% of available diversity in these crops, there is continuing need to rescue germplasm, especially wild and weedy relatives from endangered areas. NARS in developing countries also require assistance in collecting and conserving biodiversity. However, conservation is not the end by itself, but a means of making available diversity for use by the present and future generations. This requires systematic characterization and evaluation of germplasm for special traits. Traditionally, most characterization and evaluation is based on morph agronomic characters that could be easily detected and measured. However, there are many characters that are difficult to identify and are controlled by a number of genes interacting in complex ways. These include yield, time to flowering, resistance to important insect pests, diseases and abiotic stresses and quality parameters. The availability of modern biotechnological tools, particularly molecular markers, provides an opportunity to characterize germplasm for such complex traits.

Large collections of germplasm, which are difficult to handle, can be made more accessible through development of core collections, which are only 10% of entire collection but represent species diversity. ICRISAT scientists have developed core collections of all five mandate crops and finger millet. In the crops where the number of accessions is very large and core subset is also large, ICRISAT scientists have developed a strategy to select a mini-core collection (Upadhyaya and Ortiz 2001). The mini-core collection is 10% of the core collection (i.e., 1% of the entire collection) and contains almost full diversity of the entire collection. We have developed mini-core collections of chickpea (211 accessions, Upadhyaya and Ortiz 2001), groundnut (184 accessions, Upadhyaya et al. 2002) and pigeonpea (Upadhyaya et al 2006). The challenge is now to evaluate the core and mini core collections for useful traits and identify germplasm accessions as diverse parents for utilization in the breeding programs.

Wild and weedy relatives play an important role in sustaining agricultural productivity. In spite of their potential in crop breeding, very few attempts were made to utilize them for improvement of ICRISAT mandate crops, except groundnut. However, developments in biotechnology should provide new opportunities to make greater use of wild species in genetic enhancement.

This project has a global responsibility for effective protection as well as utilization of biodiversity of ICRISAT mandate crops. These primary goals will be achieved with efficient management, conservation, and the enhanced utilization of genetic resources.

Highlights of 2005

Chickpea composite collection phenotyped and genotyped

- Under the Generation Challenge Program (GCP), the chickpea composite collection (3000 accessions) was phenotyped for grain yield and related traits in an augmented design with five control cultivars during 2004-05 postrainy season. ICCs 8318, 17256, 8324, 12197, 812, and IG 70779 were the best high yielding accessions (2.74-3.35 t ha^{-1}). ICCs 12034, 13821, 16641, 17258, 5810, and ICCVs 96329 and 96030 were the earliest flowering accessions (33-36 days). ICCs 12034, 7346, and 14205 among kabuli types and ICCs 14648, 4871, and 7672 among the desi types were the largest-seeded accessions.
- The composite collection was genotyped using 35 SSRs at ICRISAT and 15 SSRs at ICARDA. Data from ICARDA is pending. The 35 SSR loci produced 1160 alleles, ranging between 15 and

58 alleles with an average of 34 alleles per SSR locus. The polymorphic information content (PIC) for the SSR loci varied from 0.47 to 0.96, with a mean of 0.84. Analyses are in progress to determine the population structure and identify the most diverse accessions for developing reference collection of 300 accessions (10% of the composite collection) for association mapping, functional genomics, gene tagging and genetic enhancement in chickpea.

Composite collections of groundnut, pigeonpea and finger millet developed

- Under the GCP, the composite collections of groundnut (850 accessions from ICRISAT and 150 from EMBRAPA), pigeonpea and finger millet (1000 accessions each) were constituted. The groundnut collection included accessions resistant to *Aspergillus flavus* seed colonization (16), various diseases (108), various insects (23) and accessions of - mini core collection (184), mini core comparators (184), mini core for Asia region (50), Asia core (60), elite/released cultivars (36), and drought tolerant (18), fresh seed dormancy (6), high and low biological nitrogen fixation (9) high shelling percentage (10), high nutritional traits (10) interspecific derivates (5), with desired agronomic traits (60) accessions genotyped earlier (18), and wilds (52) from ICRISAT, and wilds (62) and cultivated (88) from EMBRAPA.
- The pigeonpea composite collection included the mini-core collection (146), mini-core comparator (146), from core collection (236), superior morpho-agronomic traits (301), resistant to biotic stresses (74), resistant to abiotic stresses (14), elite/released cultivars (20), and 63 accessions of 7 wild species.
- The finger millet composite collection included core collection (622), plant growth aspect (114), core collection of Indian Coordinated Small Millet Improvement Project (50), desired agronomic traits (117), grain nutritional traits (12) and resistant to diseases (85). During 2006 this composite collection will be genotyped using 20 SSR markers, and the information will be used to determine population structure and identifying a reference collection of 300 accessions.

Composite collections of groundnut, pigeonpea and finger millet phenotyped

- A set of 850 groundnut accessions from ICRISAT (part of groundnut composite collection) was phenotyped for pod yield and related traits in an augmented design with four control cultivars, during 2005 rainy season. ICGs 6201, 6407, 6703, 10566 and 15042 were early flowering (19 days) in comparison to control cultivar ICGS 44 (22 days). ICGs 3027, 8352, 8285, 13920, and 13916 produced high pod yield (4.40 – 4.82 t ha^{-1}) in comparison to control cultivar ICGS 76 (4.38 t ha^{-1}) and ICGs 44 (3.88 t ha^{-1}). ICGs 5016, 4304, 8305, 8352, and 12059 were large-seeded (87 – 89 g 100 seed weight) accessions
- The pigeonpea composite collection was phenotyped in an augmented design with four control cultivars, for yield potential and related traits during 2005 rainy season. Data recording is in progress. Similarly, the finger millet composite collection was phenotyped in an augmented design with four repeated control cultivars for traits related to grain yield during 2005 rainy season. The preliminary investigation revealed that 65 finger millet accessions were promising for seed yield than the control cultivars. The early flowering accessions were IEs 4442, 4702, 6013, 588 and 4759 (42-51 days).

Composite collections of groundnut and pigeonpea genotyped

- The 850 accessions of the groundnut composite collection (ICRISAT contribution) were planted and leaf material was used to extract DNA for genotyping. Twenty SSR primer pairs were

selected at ICRISAT for pre-screening groundnut mini-core accessions to identify 10 polymorphic primers. At EMBRAPA, ten other polymorphic primers have been identified and these selected 20 SSR primers are being used to fingerprint the entire composite collection using the ABI 3700. This dataset will be used for further analysis to assess genetic diversity and then a reference collection of most diverse lines will be established that can be used for further research.

- The composite collection of pigeonpea (1000 accessions) was planted in the field and twelve plants per accession were selected for DNA extraction. Leaf samples for DNA extraction have been collected. DNA from these selected 12 plants per accession are pooled together mainly to capture within accession variation. Thirty SSR primer pairs were initially selected to pre-screen sixteen most diverse mini-core accessions to identify 20 polymorphic primer pairs, which will then be used to fingerprint the entire composite collection.

Core collections of pearl millet, pigeonpea, finger millet and foxtail millet phenotyped

- The core collections (10% of entire collection) of pearl millet (504 accessions), pigeonpea (1290), finger millet (622) and foxtail millet (155) were phenotyped during 2005 rainy season in augmented design with repeated control cultivars. The pearl millet results revealed that IP 9496 flowered in less than 45 days and IPs 10423 11937, 11947 and 17435 flowered very late (>125 days). IP 15220 and IP 10401 grew to a height of less than 40 cm. IP 15304 and IP 15257 produced 6 and 8 productive tillers. IPs 5416, 12310 and 5447 produced panicles longer than 65 cm and more than 25 mm thickness. Data processing is in progress. In pigeonpea, we selected 19 accessions in extra-early maturity group (68 days to 50% flowering, harvesting index 19.0%, shelling turnover 58%, and mean seed yield 580 kg ha^{-1}) in comparison to control ICPL 87 (DF 73, HI 17.1%, shelling 51.7%, and seed yield 219 kg ha^{-1}). Similarly, 5 early maturing, 6 medium maturing, and 29 late maturing high yielding accessions combined with other traits of economic importance were identified.
- In finger millet, IEs 2288, 3280, 3952, 5066 and 5179 (2.04-2.15 t ha^{-1}) were identified as high yielding accessions and IEs 501, 2322, 2957, 4759, and 6013 as early flowering accessions (49-52 days to 50% flowering). Similarly, in foxtail millet core collection, ISe 1254, 1227, 1234, 1286 and 1161 were early flowering (25-33 days) accessions. The preliminary investigation revealed that 16 germplasm accessions were promising for seed yield.

Evaluation of pigeonpea mini-core for pigeonpea sterility mosaic virus resistance

- Pigeonpea mini-core collection (146 accessions) was evaluated against two isolates of pigeonpea sterility mosaic virus (PPSMV), the B-isolate at Bangalore and P-isolate at Patancheru, India. Plants were inoculated with respective PPSMV isolates at 2-leaf stage, monitored for symptom type and percent incidence at 2 weekly intervals scoring was based on visual symptoms and plants were also tested for virus by ELISA using PPSMV-P polyclonal antibodies. ICP 8863 was used as susceptible control.
- On screening against P-isolate, 3 accessions (ICPs 7869, 14120 and 14155) showed no infection; 8 accessions showed 1-10% infection; and 11 accessions showed 11-30% infection. The rest of the accessions showed >30% infection. Five accessions (ICPs 12123, 10654, 11015, 11059 and 3046) although showed >80% infection, the genotypes expressed chlorotic ring spot symptoms, and no sterility observed in these genotypes and these can be regarded as tolerant to SMD. On

screening against B-isolate, four accessions (ICPs 6123, 15185, 14569 and 14976) had <30% infection. Symptoms appeared very late. It appears that resistant sources to B-type of isolates are scarce.

Trait-specific mining of novel alleles and genes in pearl millet

- During 2005, in collaboration with researchers at the International Centre for Genetic Engineering and Biotechnology (ICGEB) and the Central University of Hyderabad, an attempt was made to generate gene-based markers associated with a pearl millet drought tolerance QTL, mapping in linkage group 2, which was mapped from donor parent, PRLT 2/89-33. This was done by isolating RNA samples from flag leaves of pairs of hybrids, near-isogenic for the drought tolerance QTL allele(s), i.e., 843A × H 77/833-2 and 843A × ICMR 01029 that had received either a fully-irrigated control treatment or a drought stress treatment initiated at panicle emergence in a greenhouse dry-down experiment. Using RNA samples collected from stressed plants of the two hybrids, a subtractive cDNA library was developed. Recombinant clones were collected and EST sequences corresponding to approximately 300 unigenes obtained. Initial annotation of the EST sequences suggested some chloroplast genome contamination of the cDNA library. So far, none of the EST sequences obtained appear to contain SSRs that might exhibit polymorphism between the near-isogenic pollinator of the two hybrids. However, such polymorphic EST-SSRs (and other polymorphic EST-based markers) could be used for exploratory allele mining in the vicinity of the pearl millet drought tolerance QTL located on linkage group 2.

Assessment of genotypic diversity in a set of agronomically superior insect resistant sorghum germplasm

- During 2005, genetic diversity was re-assessed for the set of 91 elite sorghum germplasm accessions using SSR markers. The set included 12 shoot fly and 15 stem borer resistant accessions, 9 accessions resistant to both shoot fly and stem borer, 17 midge resistant accessions, and 38 agronomically elite recurrent parents for which ICRISAT previously initiated a large-scale marker-assisted backcross program for the stay-green components of terminal drought tolerance. Twenty SSR markers were used to separate the PCR products using capillary electrophoresis that generated a total of 118 alleles with an average of 5.1 alleles per SSR locus. A high level of polymorphism was detected by 13 out of 20 (65%) SSR markers. An UPGMA (un-weighted paired group method with arithmetic averages) dendrogram was then constructed using Jaccard's similarity coefficient between each pair of accessions (which ranged from 0.28 to 1.00). The dendogram showed clustering of accessions in respect to geographical origin, race and specific traits such as resistance to specific insect pests. Based on clustering pattern, it was clear that the accessions under study are not only diverse for midge, shoot fly and stem borer resistance but also for agronomically superior recurrent parents that grouped in different clusters. However, some of the accessions with resistance to midge, shoot fly and stem borer clustered separately, suggesting that these lines might contain unexploited genetic variation for insect resistance that could be further exploited in breeding programs. This information will be useful for identifying elite recurrent parents for marker-assisted backcrossing programs to introgress insect resistance QTLs from the currently available mapping populations. Further, newly identified pairs of agronomically elite and genetically diverse insect resistant breeding lines could be used for developing new mapping populations to detect additional insect resistance QTLs.

Phenotypic and molecular characterization of sorghum germplasm in East and Central Africa

- Molecular markers provide information on the global genetic structure of the species – typically by using 20-50 SSR markers – and they provide the basis for association studies and determine entry points into vast germplasm collections. Once validated, information on marker-trait associations can be used for marker-assisted breeding. Through this project, standardized documentation, phenotypic and molecular characterization of sorghum accessions held as breeding material, international nurseries or as conserved germplasm in national gene banks of 8 ECA NARS is being analyzed. A regional composite set of 200 accessions per country is being analyzed for 25 markers and the additional diversity will be readily placed in relation to the components of the global sorghum germplasm structure. Capacity in high-throughput capillary based genotyping and low-cost PAGE systems is being built.

Phenotypic and genotypic diversity assessment of nutritional quality in sorghum and pearl millet germplasm

- The phenotypic diversity for grain micronutrient density in sorghum was assessed. The genetic variability for grain densities of Fe, Zn and phytates and high broad-sense heritabilities was observed. However, there is limited variability for beta-carotene density in sorghum, and all materials with detectable levels of this micronutrient in their grain have yellow endosperm. Grain densities of Fe and Zn were positively correlated, though each one of them had negative correlation with yield.
- Similarly, phenotypic diversity assessment for grain micronutrient density in pearl millet was assessed. Although there are significant genotype × environment interaction for pearl millet grain densities of Fe and Zn, there were good correlations between two seasons [Fe ($r=0.66^{**}$) & Zn ($r=0.69^{**}$)] and genotype rankings for Fe and Zn density were fairly consistent across environments and laboratories. There was wide variability for grain Fe and Zn density and like sorghum, in pearl millet too grain densities of Fe and Zn were positively correlated. A rapid, low-cost, staining protocol of Fe grain density has been optimized and can now be used for inexpensive high-throughput screening of core collections of sorghum and pearl millet.

Information management systems

- The workflow management system (LIMS/Laboratory Information Management System) came into operation in 2005 to help with the capture of genotyping data from the Applied Genomics Laboratory (AGL). This three-tier application (data layer, middleware and presentation layer) is modular in its construction. This allows further enhancements to be made if required through the use, re-use or replacement of existing modules. LIMS also incorporates automated allele binning through a Java executable of the algorithm of Cordon and Idury (1997). Testing for user acceptability of this application is currently in progress. Genotyping information from the LIMS system flows into a larger database ICRIS (Integrated ICRISAT Crop Resources Information System). The ICRIS is envisaged to be an integrated database and currently consists of three databases for genetic resource data, genotyping data and phenotypic/trait information. The database can be accessed through the user interface, tabular and graphical reports can be generated from existing data.

Human Resource Development

A short term course on Plant genetic diversity analysis and marker-assisted breeding held at Kamphaeng Saen Campus of the Kasetsart University Thailand during 20 Aug – 04 Sept 2005. This course was funded by Generation Challenge Program. Twenty participants from 10 countries participated in the course

Several Apprentices were given hands on training on basic molecular biology/ biotechnology training through university linked apprentice program, internships.

Several Research Scholars and Research Fellows enrolled for Ph.D. work

A Bio-informatics workshop conducted during 17-21 October 2005

A three-day training course on "Designing and Analysis of Participatory On-Farm Trials held during 25-27 May 2005

Beneficiaries

National and international public and private sector breeding programs, plus the farmers who adopt new varieties developed by those programs and the rural poor who depend on these crops.

Collaborators

INERA (Burkina Faso); NARI (Eritrea); BECA (Kenya); KARI (Kenya); IER (Mali); INERA (Niger); IAR (Nigeria); ISRA (Senegal); ARTC (Sudan); Networks (CLAN, ECARSAM, ROCAFREMI, SMINET, and ROCARS); and regional fora (ASARECA, SACCAR, CORAF, and FARA); IITA, ILRI and ICRAF and LCRI (Nigeria).

Queensland Department of Primary Industries, Hermitage Research Station, University of Queensland, Melbourne University (Australia); Vrije Universitiet Brussel and Catholique University, Louvain-la-Neuve (Belgium); PBI (Canada); University of Hohenheim (Germany); IGER, JIC, SCRI, University of Birmingham, Centre for Arid Zone Studies (UK); Cornell, Purdue, Texas A&M, Washington State and North Carolina State Universities, University of California-Davis, University of Wisconsin, University of Georgia (USA); CG centers (CIAT, ILRI, IITA and ICARDA).

Linkages to CGIAR Outputs

Germplasm improvement	70%
Sustainable production systems	10%
Policy	10%
Enhancing NARS	10%

Section 2

Global Project 1

Improved abiotic stress tolerance, agronomic and quality traits via the application of genomics, genetic engineering and wide-hybridization

[Project Coordinator: PM Gaur]

Output 1.1.1 **Enhanced Drought Tolerance in Pearl Millet and Sorghum**

Activity 1.1.1.1 Marker-assisted introgression of drought tolerance QTLs in pearl millet

Team Hash CT, Vadez V, Bidinger F, Rizvi SMH, Chandra S

Milestone Root traits in LG 2 drought tolerance QTL-NIL of pearl millet characterized (2006)

The main purpose of experiments conducted in 2005 was to test whether roots have any role to play in the QTLs identified for terminal drought tolerance in pearl millet (QTLs that were detected for differences in panicle harvest index, PNHI, under conditions of terminal drought stress). We used four contrasting parents (drought sensitive H 77/833-2 and 841B, and drought tolerant PRLT 2/89-33 and 863B), plus two QTL-NILs in which a major drought tolerance QTL from LG 2 has been introgressed from donor parent PRLT 2/89-33 into the genetic background of H77-833/2 via several cycles of marker-assisted backcrossing (ICMR 01029 and ICMR 01031).

In a first experiment, we grew plants in 18 cm diameter 120 cm deep PVC cylinders, during September-December 2004. Twenty replicated plants were grown for each genotype, under well-watered conditions for the first 2 weeks after sowing. Thereafter, half of the plants were exposed to water stress by withholding irrigation while the other half was kept under well-watered conditions (control). One set of plants (5 replicated plants per genotype in stress and control) was harvested at 35 DAS (days after sowing) and the second set was harvested at 67 DAS. The second experiment was planted during March-April 2005. This experiment was basically a repeat from the first experiment, except that cylinders used were 200 cm long (same diameter), the second harvest was done at 63 DAS, and the genotypes evaluated included inbred lines 841B and 863B, the parental lines of a second pearl millet terminal drought tolerance mapping population. At harvest, the entire root system was gently pulled out after washing away the soil, its entire length measured and thereafter sliced in 30 cm portions to measure dry weight and root length density of the different portions.

Data of the first and second experiments were very consistent with each other, despite the differences in cylinder length, and therefore results of the second experiment are discussed. In pearl millet, roots of tolerant genotypes reached a length of up to 240 cm in the second harvest in those plants exposed to water stress, whereas no sensitive pearl millet genotype had roots longer than 210 cm under water stress. We also looked at the root dry weight in each of the 30 cm layers. We found that all 4 pearl millet genotypes having tolerance to terminal drought had also larger "investments" in deeper rooting than the drought sensitive pearl millet genotypes. Expressed as a percentage of roots in the 0-30 cm layer (to compare to shallow rooting), tolerant pearl millet genotypes had 15-20% of the roots present in soil layers deeper than 150 cm, whereas sensitive pearl millet had less than 5% of roots deeper than 150 cm.

These results are very encouraging as they show a significant and consistent involvement of roots in the terminal drought tolerance QTLs of pearl millet. The protocol used to pinpoint these differences can be further refined.

Vadez V

Milestone The leaf gas exchange response (photosynthesis, Gs) to drought and TE assessed in LG 2 drought tolerance QTL NIL of pearl millet (2006)

One experiment was carried out to compare the response to soil drying and transpiration efficiency of various parental lines of pearl millet genotypes contrasting for terminal drought tolerance. Pearl millet genotypes used were ICMR 1029, ICMR 1031, H 77/833-2, and PRLT 2/89-33. In this experiment, carried out during October-November 2004, we found that pearl millet genotypes differed very little for the threshold of soil moisture where transpiration starts to decline. We also measured transpiration efficiency in that experiment and found that all the genotypes had very similar TE.

Vadez V

Milestone Products of markers-assisted backcrossing (MABC) for drought avoidance QTLs tested in pearl millet (2006)

Products of MABC were advanced by one generation for development of drought tolerance QTL-NILs in the background of elite maintainer line 841B having introgressed alleles from LG 1 and LG 6 of donor 863B. Products of MABC for drought tolerance QTL-NILs with LG 2 substitutions from donor 863B in the background of elite maintainer line 841B were tested in second year. We conducted the fourth year of testing of products of MABC for drought tolerance QTL-NILs with LG 2 substitutions from donor PRLT 2/89-33 in the background of elite restorer line H 77/833-2. Products of MABC for drought tolerance QTL from LG 2 of donor PRLT 2/89-33 in background of elite pollinator H77/833-2 were retested. Obtained special project funding extension of 10 months to continue research in this activity area (supported by the DFID PSP pearl millet marker-assisted breeding project). The project activities are being conducted with national program partners. Six new CAZRI drought tolerance populations were advanced to F_2/BC_1F_1 population pairs and sent to CAZRI and the AICPMIP.

Hash CT, Senthivel S , Satish Kumar P and Rizvi SMH

Activity 1.1.1.2 Marker-assisted breeding for stay-green QTLs in sorghum

Team Hash CT, Vadez V, Bidinger F, Rizvi SMH, Folkertsma R, Mgonja M, Rattunde F, Chandra S

Milestone Techniques standardized for large-scale screening of root traits in sorghum.

The main purpose of experiments conducted was to test whether roots have any role to play in the QTLs thus far identified for terminal drought tolerance in sorghum (QTLs that were detected for the stay-green component of terminal drought tolerance). We used two elite drought-sensitive parents [ISIAP Dorado (partially senescent) and R 16 (highly senescent)], two stay-green donor parents (B35 and E 36-1), and two derivatives of R 16 thought to carry 2 or 3 major stay-green QTLs (RSG 03123 with drought tolerance from donor B35 and RSG 04012 with drought tolerance from donor E 36-1).

In the first experiment, we grew plants in 18 cm diameter and 120 cm deep PVC cylinders, during September-Dec 2004. Twenty plants were grown for each genotype, under well-watered conditions for the first 2 weeks after sowing. Thereafter, half of the plants were exposed to water stress by withholding irrigation while the other half was kept under well-watered conditions (control). One set of plants (5 replicated plants per genotype in stress and control) was harvested at 35 DAS and the second set was harvested at 67 DAS. The second experiment was conducted during March-April 2005. This experiment was basically a repeat from the first experiment, except that cylinders used were 200 cm long (same diameter), and the second harvest was done at 63 DAS. Genotypes ISIAP Dorado, E 36-1, and RSG 04012 were included in the second experiment. At harvest, the entire root

system was gently pulled out of the cylinder after washing away the soil, its entire length measured and thereafter sliced in 30 cm portions to measure dry weight and root length density in these different portions.

Data of the first and second experiments were very consistent with each other, despite the difference in cylinder length, and therefore results of the second experiment are discussed. We found that roots of stay-green trait donors and stay-green QTL introgressed materials reached all a length of at least 270 cm in the second harvest under water stress, whereas senescent genotypes ISIAP Dorado and R 16 had a maximum root length of 210 cm. Again, expressed as percentages of roots in the 0-30 cm layer (to compare to shallow rooting), stay-green sorghums had 15-35% of the roots present in soil layers deeper than 150 cm, whereas senescent sorghums had only 5-10% of roots deeper than 150 cm. Interestingly, stay-green and senescent materials did not vary for root length under well-watered conditions.

These results are very encouraging as they show a significant and consistent involvement of roots in the stay-green QTLs associated with terminal drought tolerance in sorghum. The protocol used to pinpoint these differences can be further refined. As an attempt to do so, a third experiment was started with sorghum, where stress was imposed at 21, 35, 49, and 69 (flowering) days after sowing. At each date of stress imposition, one set of plants (5 replicated cylinders per genotype) was pre-harvested and two additional sets were submitted to drought and well-watered conditions. Harvest of the two sets undertaking treatment was done once wilting symptoms were visible on all plants, usually 3-4 weeks after treatment imposition.

Vadez V

Milestone — First products of marker-assisted backcrossing of stay-green QTL from donor E 36-1 into the backgrounds of S35 and IRAT 204 field-tested to assess terminal drought tolerance (2008)

We evaluated sets of progenies from MABC introgression of several putative stay-green QTL into released cultivars ISIAP Dorado (BC_3F_4 lines, with donor parent B35) and R 16 (BC_1F_3 lines, with donor parents B35 and E 36-1) in both supplementally irrigated and dryland environments in the postrainy season of 2004-2005. The objectives were to assess the expression of stay-green in the backcross progenies under postrainy season conditions, to compare the agronomic expression of the recurrent parents and their backcross derivatives, and to evaluate the effects of stay-green expression on grain filling and grain yield under the characteristic post-flowering drought stress of the postrainy season. Environmental (moisture) differences in stay-green expression were strong in both sets of materials; genetic differences in stay green expression were clear in the case of the highly senescent R 16, but less so in the case of the moderately senescent ISIAP Dorado. For example, the percent green leaf area 2/3 of the way through grain filling in the R 16 derivatives ranged from 7% to 29% in the dryland environment, compared to 34% to 57% in the ISIAP Dorado trial (in which stress was interrupted by a rain shower in mid grain filling). None of the backcross progenies expressed the stay-green trait to the same degree as the donor parent B35, but most have only one or two stay-green QTL, in contrast to B35, which has a larger number (six or more).

Many of the R 16 derivatives differed agronomically from the recurrent parent in height and flowering time, and some in grain size and stover yield. The majority were similar in grain yield to R 16 in both environments. However, most of the differences reflect the differences in the donor parents and R 16, and should disappear with further backcrosses. The derivatives of ISIAP Dorado were generally more similar to the recurrent parent (as would be expected from BC_3 materials), except for later flowering, and some tendency for the derivatives to have a greater stover yield and a smaller grain size.

Stay-green expression during the second half of the grain filling period was related to relative (dryland environment/supplementally irrigated environment) grain size *(i.e.*, to completeness of grain filling) in both sets of materials: $r^2 = 0.61$ ($P < 0.01$) for R 16 (Figure 1.1) and $r^2 = 0.59$ ($P<0.01$) for ISIAP Dorado. The effect of differences in stay-green expression on grain size carried over into a significant effect on grain yield in the case of R16 ($r^2 = 0.48$) but not in the case of the ISIAP Dorado derivatives ($r^2 = 0.10$). We hypothesize that the difference in stay green effect in the two trials was due to both genetic (R16 is normally considerably more senescent than is ISIAP Dorado) and environmental (the rain shower during mid grain filling in the ISIAP Dorado trial) reasons. Selected R 16 evaluation will be repeated in 2005-2006, and better R 16 derivatives from 2004-2005 trial were backcrossed to R16 twice during 2005 prior to possible re-evaluation during 2006-2007.

Bidinger FR and Hash CT

Milestone — Sorghum stay-green introgression lines derived by marker-assisted backcrossing provided to African national programs in locally adapted, farmer-preferred genetic backgrounds (2008)

Due to lack of adequate SSR marker polymorphism between stay-green drought tolerance donor parent E 36-1 and the two recurrent parents (S35 and IRAT 204), and reduction in the amount of funding provided by granting agencies for activities related to this milestone, we have focused the marker-assisted backcrossing program for the sorghum stay-green trait on donor B35 only.

During 2004 marker-assisted backcrossing of genomic regions associated with the stay-green component of terminal drought tolerance of donor parent B35 was advanced two generations in the genetic background of elite recurrent parent Kapaala = ICSV 111. By the end of 2004, BC_4F_2 seed

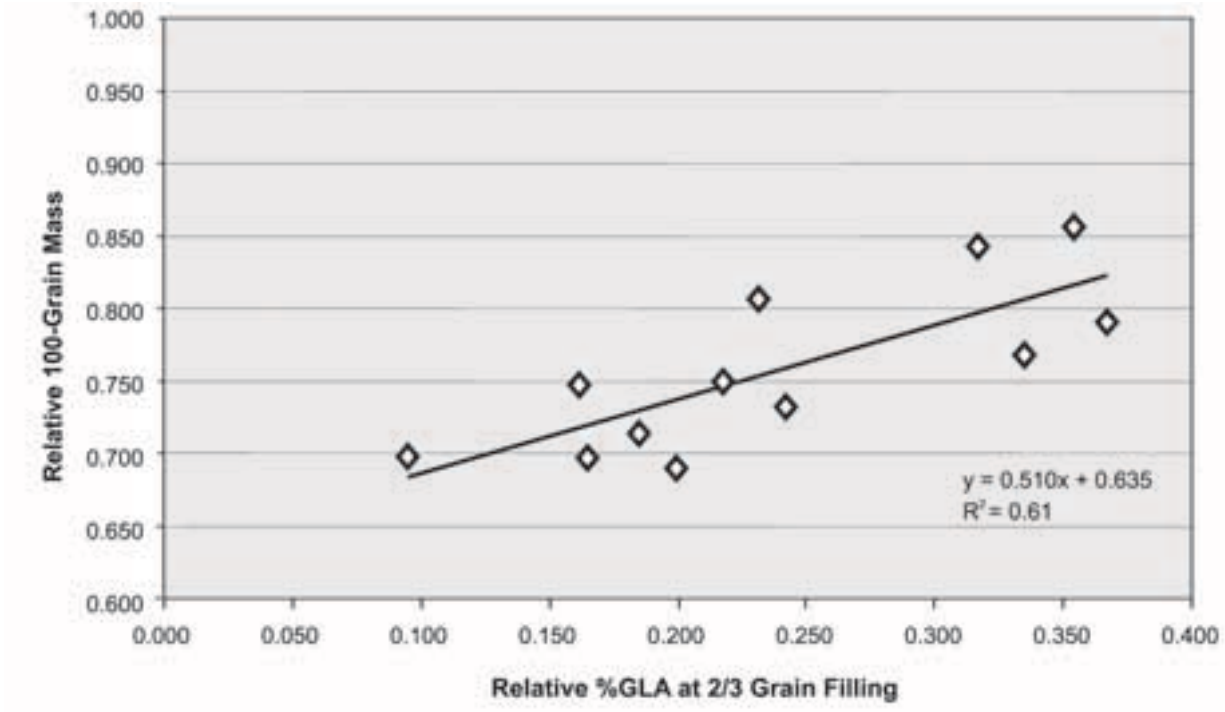


Figure 1.1. Relative (dryland/supplementally irrigated environments) 100-grain mass as a function of relative (dryland/supplementally irrigated environments) percent green leaf area (%GLA) at two-thirds of the way through grain filling for R 16 and its derivatives.

had been produced for families expected to segregate for four of the six target stay-green QTL in this genetic background (for which suitable BC_3F_1 families were available in 2003) and for the remaining two target QTL, seed had been produced for BC_3F_2 families.

During the first half of 2005, BC_3F_2 and BC_4F_2 plants homozygous for donor parent alleles at SSR marker loci flanking each of six individual stay-green loci (*i.e.*, single-QTL introgression lines for stay-green QTLs *stg*A, *stg*B, *stg*1, *stg*2, *stg*3 and *stg*4) from donor parent B35 in the genetic background of ICSV 111 (released in Ghana as 'Kapaala') and its sub-selection S 35, were identified and their selfed seed harvested. These included ten BC_4F_2 plants expected to be homozygous for *stg*A, three BC_4F_2 plants expected to be homozygous for *stg*B, six BC3F_2 plants expected to be homozygous for *stg*1, ten BC_3F_2 plants expected to be homozygous for *stg*2, two BC_3F_2 and seven BC_4F_2 plants expected to be homozygous for *stg*3, and four BC_4F_2 plants expected to be homozygous for *stg*4. The corresponding BC_4F_3 and BC_3F_3 families, along with seed samples of their recurrent and donor parents, were sent to the national sorghum breeding program in Ghana (SARI) in June 2005 in time for initial rainy season seed increase for agronomic and farmer-participatory evaluation under the Water for Food Challenge Program. Further, these progenies were advanced a further generation by selfing at ICRISAT-Patancheru during the 2005-06 post-rainy season to produce seed required for future assessment of their drought tolerance, agronomic performance and grain quality. Marker data generation in 2005 required to identify the putative QTL introgression homozygotes was performed as part of the M.Sc. thesis research program of Mr. Sripathi Venkateswararao. In late 2005 he submitted a thesis to Acharya N.G. Ranga Agricultural University (ANGRAU) India, and this was defended successfully in early 2006.

Similarly, marker-assisted backcross introgression of stay-green QTLs from donor parent B35 into the genetic background of recurrent parent IRAT 204 advanced two generations during 2005, with the marker data generation required being performed as part of the M.Sc. thesis research program of Mr. Sripathi Venkateswararao.

We hosted a Visiting Scientist from the Indian national program (Dr Madhasudhana Rao from the National Research Center for Sorghum) for 3 months to develop marker genotype data for assessing opportunities of MABC to improve stay-green drought tolerance of elite and landrace materials of interest to Indian sorghum breeders, and another from the Universidad Autonoma de Neuvo Leon (Dr F Zavala G) for 1 month to learn how to conduct SSR-MAS for the stay-green trait.

Hash CT

Output 1.1.2	**Enhanced Drought Tolerance in Chickpea and Groundnut**
Activity 1.1.2.1	Marker-assisted breeding for drought-avoidance root traits in chickpea
Team	Gaur PM, Vadez V, Kashiwagi J, Varshney RK, Hoisington D, Chandra S
Milestone	Markers for drought-avoidance root traits in chickpea identified and verified in unrelated populations (2006*)*.

***Drought avoidance root traits in chickpea further dissected*:** Lot of knowledge on root traits in chickpea has been gathered in the past two decades. However, we have little understanding of how roots work and how much and at what stage deep rooting actually contribute to water extraction, in particular for conferring tolerance to terminal drought. Two experiments were designed to start addressing this issue, using three genotypes with deep and profuse rooting (ICC4958, Annigeri and ICC8261), and two genotypes with shallow rooting (ICC1882 and ICC283). Plants were grown in cylinders made of assembled 30 cm portions of 10 cm diameter PVC tubes. Plants were grown under

well-watered conditions for the first two weeks after sowing, after that irrigation was withdrawn in half of the tubes. Harvests were performed at different times after stress imposition and each time, tubes were split into 30 cm portions (0-30, 30-60, 60-90, and 90-120). Roots in each portion were separated. After separation, soil was homogenized quickly and a soil aliquot was taken, weighted, dried at 80ºC for two days and weighted again to determine water content. The main purpose of the experiment was to relate root length density in the different cylinder portions to water content remaining. Preliminary interpretation in the first experiment shows that deep rooted genotypes extract more water from the 60-90 cm layer at 25 DAS, and, the rooting profile moving further down, thereafter extract more water from the 90-120 cm layer at 34 DAS, than shallow rooted genotypes. These results need to be confirmed and refined but they have the potential to greatly simplify the screening of root traits. One particular interest of this technique would be to study how water uptake by deep rooting plants contributes to providing water at that time of seed filling.

Vadez V

***Estimation of gene effects for drought avoidance root traits*:** The genetic components of root characteristics were investigated through generation mean analysis, using the means of six basic populations (P_1, P_2, F_1, F_2, BC_1, and BC_2) of two crosses (ICC283 × ICC8261 and ICC4958 × ICC1882). The additive gene effect played important role in controlling root length density (RLD) in both the crosses. Although the magnitudes of additive gene effects and additive × additive gene interactions of total RLD were different between two crosses, the direction of the gene effect was consistent towards increasing RLD in both the crosses, except 30-60 cm and 60-90 cm depth in ICC4958 × ICC1882. Although the magnitude of additive gene effect for RLD was relatively small, the cross ICC283 × ICC8261 was better than ICC4958 × ICC1882 to use in selection for larger total RLD, especially RLD in 30-60 cm depth, which is important for the seed yield under the terminal drought. The cross ICC283 × ICC8261 also showed substantial additive and additive × additive epistasis for the RLD in deeper soil section, which have greater impacts on yield under severe terminal drought. Recurrent selection in later generations would be effective for developing chickpea genotypes with higher root length density from the cross ICC283 × ICC8261 because of the relative amounts of dominance and epistasis effects.

Kashiwagi J, Gaur PM and Chandra S

***Phenotyping of new mapping population for drought-avoidance root traits*:** A RIL mapping population developed from the cross ICC4958 (large roots) × ICC1882 (small roots) was evaluated for root traits. The plants were grown in cylinder culture systems and the root traits were studied at 35 days after sowing. The root system of each entry was divided into 4 pieces in different soil depth (0-30 cm, 30-60 cm, 60-90 cm, and 90-120 cm) and scanned to obtain the digital images. Later the dry weight was recorded. All digital images were analyzed using the image analysis software WinRhizo. The parental lines are being screened for identification of polymorphic markers.

Kashiwagi J, Gaur PM and Varshney RK

Activity 1.1.2.2	Marker assisted breeding for water use efficiency in groundnut
Team	Nigam SN, Upadhyaya HD, Vadez V, Hoisington D, Varshney RK, Chandra S
Milestone	QTLs conferring water use efficiency identified in groundnut (2007)

***Phenotyping of mapping population for TE*:** A repeat of the phenotyping experiment carried out in 2004 was performed during the January-March period. The two parents TAG24 (low TE) and ICGV86031 (high TE) were tested along with 318 RIL F8 progenies from their cross. Experimental

design used was a 8 × 40 lattice design with 5 replications. Data have also been sent to the biometric unit for spatial analysis and control whether unknown patterns of variation are likely to occur in the experiment. Another similar set of plants was cultivated to evaluate the pre-treatment biomass. Preliminary analysis has been done and shows a good correlation with data gathered in 2004. This was so despite the range of TE values obtained in 2005 (1.9 – 2.4) were far less than the range of values obtained in 2004 (2.5 – 3.7). This was probably related to the fact that a late rain (45 mm) came and refilled the field capacity a few days only before planned harvest. Plants recovered from drought and this may have contributed to those being the most susceptible to intermittent drought and having low TE. In 2005 we also received the $\Delta^{13}C$ data from the 2004 phenotyping experiment and found the well-reported negative correlation between TE and $\Delta^{13}C$.

Vadez V

***Genotyping of mapping population with SSR markers*:** Parents (TAG24 and ICGV86031) of the RIL population phenotyped for WUE in groundnut were screened with 463 SSR markers. Thirty-five markers were found to be polymorphic and were screened on 318 RILs. Another 450 SSR are available and will be screened in 2006.

Varshney RK

***New sources of variability for drought tolerance traits*:** Evaluated 18 groundnut accessions with 7 control cultivars in a lattice design during 2004-05 post-rainy season, for pod yield potential and other traits related to drought. ICGs 5745, 6766, 7243, and 14523, ICG 14475 (129-150 and 132-153 Specific Leaf Area (SLA) at 60 and 80 days after sowing (DAS) and 42-44 SPAD Chlorophyll Meter Reading (SCMR) at both DAS) were identified as additional sources related to drought tolerant traits in comparison to control CMSG 84-1 (144 and 150 SLA and 43 and 42 SCMR at 60 and 80 DAS, respectively). ICGs 2773 and 5827 (3.39-3.43 t ha^{-1}) produced greater pod yield than the high yielding control cultivars ICGS 44 (2.87 t ha^{-1}) and ICGS 76 (3.21 t ha^{-1}). Leaf samples from these 18 accessions and control cultivars have been collected for DNA extraction. Finger printing using 20 SSR markers is in progress.

Groundnut composite collection consisting of 850 accessions was evaluated in an augmented design with four repeated control cultivars for pod yield potential and drought related traits. SCMR and SLA data was recorded on these accessions of groundnut composite collection at 60 and 80 DAS. ICGs 2741, 5725, 5728, 6323, and 7878 were identified for high SCMR (53.9 – 61.0). Leaf samples have been collected for DNA extraction. Finger printing using 20 SSR markers is in progress. Data for other agronomic traits is in process.

Upadhyaya HD

Activity 1.1.2.3	Transgenic drought tolerance in chickpea and groundnut
Team	Sharma KK, Vadez V, Gaur PM, Nigam SN
Milestone	Selected transgenic lines of chickpea and groundnut characterized for drought tolerance traits (2005*)*

Transgenic chickpea (P5CSF) characterized under drought: Seven events of C235 chickpea genotypes transformed with P5CSF gene were tested in our common dry-down protocol, in which we measured transpiration efficiency (g biomass per kg of water transpired, TE), and the soil moisture level (measured with the fraction of transpirable soil water, FTSW) where transpiration declines.

Growth was slightly but significantly retarded in all the transgenic events at the time of pre-treatment harvest (about 30 DAS), whereby shoot dry weight of transgenic was in the range of 1.40-1.80 g plant^{-1}, compared to 2.45 g plant^{-1} in C235. This trend was maintained at the end of the experiment under well-watered conditions where all transgenics kept a lower biomass (4.2 – 5.9g) than wild type C235 (6.6g). Under water stress, the final biomass was also somewhat higher in C235 (3.85g) than in all trangenics except one (3.20-3.55), although the transgenics had performed relatively better under drought than under well-watered conditions. This was consistent with the higher cumulative transpiration values in all the transgenics than in C235 wild type under water stress. This result fully agrees with the osmotic adjustment hypothesis, which predicts that increased turgor would help stomata to remain open to maintain transpiration rate high. It also agrees with the finding that the total transpirable soil water (TTSW), i.e. the amount of water that plants were able to extract from the soil, was higher in all the P5CSF transgenics than in the wild type under water deficit.

Although we found significant differences in TE, we found no advantage of P5CSF for TE compare to the wild type in any of the 7 events of P5CSF that were tested. All the TE values were in a narrow range of 2.10 – 2.80 g biomass kg^{-1} water transpired in well-watered plants and C235 had the highest values (2.80 g kg^{-1}). In agreement with this, we found large differences in the stomatal conductance (Gs) under well-watered conditions, with P5CSF transgenics having 3-4-fold higher Gs than C235. This correlated well with leave temperature, which was lower in transgenics than in C235, and with TE (R^2 = 0.75), whereby lower Gs correlated with higher TE, in agreement with the theory. Under water stress, we found that all the transgenics had a later initiation of stomatal closure compare to C235. TE values were in the range of 2.70 – 3.70 g kg^{-1} and C235 was in the middle of that range.

P5CSF is supposed to enhance proline production and confer drought protection of major physiological processes. We measured proline in drought stress plants but found very little differences between C235 and the transgenics. Confirmation measurements are currently being re-assessed as there was a doubt about the quality of the standards being used. We also hypothesized that the transgene contributing to osmoticum production may help keeping root growth active by maintaining turgor in root tips. Results were contrary to our expectation since C235 had larger roots than all transgenics both under well watered and water stress. However, root mass was relatively less reduced under drought stress compare to C235, indicating that root growth was somewhat improved in transgenic P5CSF under water stress.

Although there was no advantage of P5CSF for TE, the differences found in TE and in a number of aspects such as Gs, TTSW, etc. make the P5CSF transgene very interesting material to investigate certain important physiological mechanisms such as the process of water extraction by roots, or TE.

Vadez V

***Transgenic groundnut (DREB1A) characterized for leaf gas exchange and TE*:** Two experiments using 5 transgenic groundnut lines from parent JL24, inserted with the DREB1A gene, plus JL24 have been tested for the response of transpiration to progressive soil drying. These 5 events have been chosen from a preliminary screening of 14 events + wild type JL24, in which a dendrogram based on the number of days to reach FTSW=0 and the FTSW threshold where transpiration declines. From this dendrogram, we have selected one event out of the 5 main clusters, therefore choosing events ranging from very similar to very different from the wild type. This is a major contrast with earlier transgenic studies where preference is given for transgenics having an extremely contrasting phenotype compare to the wild type.

Results are very promising for several reasons: (i) The transgenic lines RD2 shows a significantly lower FTSW threshold where transpiration declines than JL24; (ii) The transpiration efficiency of this line is circa 50% higher than JL24; (iii) The TE of most transgenic lines is higher under well-watered conditions and it seems to correlate with different stomatal behavior, i.e. stomatal conductance (Gs) was lower in transgenics, in full agreement with the theory explaining differences in TE; (iv) There was a close correlation (R^2=0.87) between the FTSW threshold where transpiration begins to decline and TE, i.e. genotypes with more conservative behavior and early stomatal closure, like JL24, had lower TE than lines having late stomatal closure, like transgenic line 2; (v) There was no correlation between TE and $\Delta^{13}C$. We do not have a clear explanation for this, even after consulting the experts in that domain. However, we interpret that the lack of relation could be explained by differences in the midday stomatal opening, whereby transgenics might close up stomata during the period of maximum VPD (usually 12noon-3pm) more so than JL24, thereby saving water. This behavior might be sufficient to have better water use, while not long enough to materialize in measurable difference in the $\Delta^{13}C$ discrimination ratio.

With the range of variation in TE obtained with transgenic lines, these data give new ground for exploring the physiological basis for high TE. We have gathered such preliminary data. We are now planning new experiments to: (i) document the root behavior of transgenic plant under stress; (ii) compare TE in the most promising transgenics to that of the best germplasm accessions available so far; (iii) study the stomatal behavior of transgenics under drought; (iv) study the recovery from stress between the transgenics and the wild type; (v) prepare a first evaluation of these transgenics for yield under drought in order to relate how their performance in TE, one of the component of the yield architecture, actually convert into yield.

Vadez V

Output 1.1.3	**Enhanced Salinity Tolerance**
Activity 1.1.3.1	Mapping salinity tolerance in pearl millet and sorghum
Team	Hash CT, Vadez V, Rai KN, Reddy BVS, Chandra S
Milestones	Mechanisms of tolerance to salinity in sorghum and pearl millet identified (2005)
	Contrast for salinity tolerance between parents of pearl millet mapping populations confirmed (2005).

We confirmed previously observed contrasts for salinity tolerance using a set of sensitive and tolerant pearl millet and sorghum genotypes. Results agreed well with previous trials. In this trial, carried out in the greenhouse during a time of the year where vapor pressure deficit (VPD) is low, we found that the ratio of biomass achieved under salinity to that of control was about 40-50%, in contrast to about 10% in a previous trial conducted in outdoor conditions with these same genotypes, in a season with much higher evaporative demand (April-June). These results show the necessity to take careful consideration of the VPD while doing salinity response experiments.

A set of pearl millet breeding lines was tested again to confirm the salinity tolerance in some of them. In this trial, we also wanted to compare the evaluation for maximum forage production at booting stage, and the grain production at maturity, as it seems from earlier dataset that there is a poor correlation between booting stage evaluation and grain yield. We found a fairly good correlation between the biomass production at booting stage and the grain and fodder yield production at harvest, indicating that harvest at booting would suffice for screening pearl millet germplasm.

The 24 parental lines of 12 existing ICRISAT pearl millet mapping populations were further assessed under salinity and we have identified three contrasting pairs of parents, which will be used to identify QTL associated with salinity tolerance in pearl millet. Our activities will now focus on identifying a suitable pollinator.

A set of sorghum breeding material is also under evaluation to confirm the salinity tolerance of some material. As for pearl millet, we also would like to compare the evaluation at booting stage and that at maturity. This experiment is currently underway.

Vadez V

***Mapping QTLs for salinity tolerance in pearl millet*:** The PhD thesis research program of Ms Rupasree M advanced, assessing opportunities to exploit existing pearl millet mapping populations for mapping components of salinity tolerance in pearl millet. She was able to develop and map over 40 TRAP markers based on gene sequences for three enzymes expected to be involved in salinity stress tolerance, and to phenotype the mapping population in which these were mapped for salinity tolerance during germination and early seedling growth, and map a few QTLs for these under control and salinity stress (150 mM NaCl) conditions. The data analysis is on-going.

Hash CT

***Assessment of molecular diversity in a set of sorghum germplasm with variable tolerance to salinity*:** SSR-marker-based genetic diversity assessment was initiated for of a set of 30 sorghum genotypes identified as either sensitive to salinity stress under pot and field conditions (5 entries) or as tolerant to salinity stress under pot conditions and productive under saline field conditions (25 entries). Initial results, based on information generated using 76 SSR marker primer pairs, indicate 1) that there is substantial genetic diversity within the range of productive and apparently salinity tolerant breeding lines and elite varieties tested, 2) that sufficient marker polymorphism is available to allow genotyping of mapping population progeny sets that could be produced by crossing selected pairs of salinity sensitive and apparently salinity tolerant genotypes, and 3) that there is surprising similarity the marker genotype level (apparently identical PCR products for 63 of 75 tested SSR primer pairs) between elite varieties S 35 and NTJ 2, both of which were selected as productive under saline field conditions, and which apparently share common '*zera-zera*' landrace germplasm in their ancestry. Based on these results, crossing necessary to initiate formation of new sorghum mapping populations specifically targeting salinity tolerance can begin.

Hash CT

Activity 1.1.3.2	Mapping salinity tolerance in chickpea, groundnut and pigeonpea
Team	Vadez V, Gaur PM, Nigam SN, Saxena KB, Upadhyaya HD, Mallikarjuna N, Hoisington D, Chandra S
Milestones	Salinity tolerant lines identified in chickpea, groundnut and pigeonpea (2005) Mechanisms of tolerance to salinity identified in chickpea groundnut and pigeonpea (2006).

***Chickpea*:** A large collection of chickpea genotypes was screened for salinity tolerance. Four sets of plants were used. Two sets were grown in 10.5" pots filled up with 10 kg black soil, treated with either 0 or 80 mM NaCl solution at sowing (sufficient to saturate the field capacity), thereafter watered with soft water up to maturity. Two other sets were planted in 6.5" pots containing 3 kg of soil, treated with either 0 or 100 mM NaCla solution at sowing, thereafter watered with soft water up to harvest

at 50 DAS. The set of 272 genotypes included the entire mini-core collection of ICRISAT, breeding lines, wild derivatives of chickpea, and all chickpea genotypes reported in the literature as being tolerant to salinity or sodicity.

There was a good correlation between the biomass data obtained at 50 DAS under salinity and those obtained with 252 common genotypes in 2004, indicating that our screening facility provides good and reliable data.

Although it has been reported several times in the past that there was little genotypic contrast in chickpea for salinity tolerance, we found over a 6-fold range of variation in the seed yield per pot. In particular, we found that 6-8 genotypes had a 20% higher seed yield under salinity than the best released variety for salinity tolerance, CSG8962. We also tested the parents of existing mapping populations. We found that ICCV2 and JG 62 were among the most contrasting genotypes for seed yield under salinity, with ICCV2 being the most sensitive whereas JG62 was among the most tolerant.

Investigation of possible tolerance mechanisms was investigated. We found a good correlation between seed yield under salinity and seed yield under control, showing that yield potential was partially determining the yield under saline conditions. Sodium was analyzed in shoot tissue and showed that there was a negative correlation between biomass and Na accumulation. However, there was no significant correlation between shoot Na accumulation and seed yield. We found that desi type were usually more tolerant than Kabuli types to salinity. We found that seed yield per pot was well correlated to the number of pods per plants under salinity but poorly correlated to the seed size, showing that salinity effect played a role probably more during the pod setting and/or grain formation that during seed development and filling. We also found that maturity was significantly related to salinity tolerance, in a form of an inverse parabola. Extra early genotypes had fairly low seed yield under salinity. There was an extremum flowering time for salinity tolerance of about 54 DAS, after which there was a sharp decrease in seed yield of later genotypes. Last but not least, we did not find any correlation between the biomass at 50 DAS and the seed yield at maturity, meaning that subsequent screening for salinity tolerance in chickpea need to be done up to yield.

***Groundnut*:** A repeat experiment using 6 genotypes was carried out to finalize the standardization of the screening protocol. It showed that an application of a 100-125 mM NaCl solution to saturate the field capacity of Alfisol was a suitable treatment to find good phenotypic contrast in groundnut. In particular, we found that tolerant groundnut keep the ability to expand leaves. By contrast, there seemed to be no relation between the biomass produced under salinity and the accumulation of Na. As in sorghum, it seems that groundnuts are able to accumulate large amounts of Na in the stem. Whether this could be used as a screen for salinity tolerance has not been tested.

Based on this protocol, a large screening was performed in the salinity screening facility in outdoors conditions. A total of 288 genotypes were tested, including the entire mini-core, breeding lines, as well as genotypes that we selected based on their origin (passport data) from the Chaco area from North Argentina, South-East Bolivia and West Paraguay, an area known to be affected by soil salinity. The screening was carried out during the April-June period. Plants were grown in 10.5" pots filled up with 10 kg black soil, treated with either 0 or 100 mM NaCl solution at sowing (sufficient to saturate the field capacity, but applied in three split doses during the initial 12 days after sowing). Because other experiments were planned at the same time in the screening facility, we could only evaluate the yield by the pod number at harvest (60 DAS). Nevertheless, we found here also a 5-6-fold variation in pod numbers across genotypes. Breeding line ICCV92206 was among the best. In peanut also we

found a poor relation between pod number and seed yield. A repeat of this experiment is foreseen for early 2006, in which we expect to measure yield.

***Pigeonpea*:** Same as in groundnut, a repeat experiment was done to finalize the standardization of the screening protocol, whereby 75 mM NaCl turned out to be the most suitable treatment to reveal genotypic contrast in pigeonpea. Interestingly, we found that SCMR reading could be a good screen for salinity tolerance in pigeonpea.

Large screening was carried out for salinity tolerance in pigeonpea. Pigeonpea is among the legumes that are the most sensitive to salinity stress. In the 1990's several papers have come up showing the relative tolerance of wild relatives of pigeonpea such as C*ajanus platycarpus*, and C. *sericeus*. The purpose of this initial large screening was to probe the range of variation in the response to salt stress using a large range of material from the germplasm bank. This range included the newly set up mini-core collection of pigeonpea, wild relatives *acutifolius*, *platycarpu*s, *sericeus*, *albicans*, etc., wild derivatives from the breeding program, and a few hybrids. In this we also made an effort to select pigeonpea having differences in crop duration (from short to long duration). We found large contrast for biomass production at vegetative stage (biomass at 50 DAS), with large contrast between accessions of C. *scaraboides* and of C. *sericeus*. This opens the possibility to develop mapping populations of wild relatives.

Vadez V

Output 1.1.4	**Enhanced Nutrient Uptake Ability**
Activity 1.1.4.1	Marker-assisted breeding for phosphorus acquisition ability in pearl millet
Team	Hash CT, Vadez V, Chandra S
Milestone	African pearl millet germplasm identified for P acquisition from poorly soluble P sources and development of mapping populations for QTL of P acquisition ability initiated (2006)

***Mechanisms of low P acquisition*:** We have now set up a protocol in the lab to measure root acidification of pearl millet seedling. This protocol combines pH measurement and image analysis and could potentially be used for large screening. It basically consists of using the colorimetric relation with pH of a colour indicator. Plantlets are inserted in a 3 mm layer of 0.9% agar + bromocresol blue. Image of the plate are scanned at regular interval during 24 hours. Agar is then melted and changes in color measured spectrophotometrically at 540 nm. Alternatively, and in a more precise way, the scanned images are converted in layers of different color, *i.e.*, different pH values. The respective surfaces are measured and H^+ extrusion calculated. This allows mapping of the H^+ extrusion. With this technique, we can potentially screen the parents of existing mapping populations of pearl millet. The current orientation of this work is to modify this method to make measurement of acidification in older plants and correlate results with measurements at the seedling stage.

Phenotyping of low P millet: The phenotyping of the testcross hybrids obtained from the combination of four different male-sterile lines and the mapping progenies of the crosses between 81B-P6 and ICMP 451-P8, and between LGD 1-B-10 and ICMP 85410-P7 was undertaken in February 2005. This was the first time that scaling up low P screening was undertaken for pearl millet at ICRISAT-Patancheru. The experimental design used was an alpha lattice with 5 replications, 1 pot per entry per replication, and 1 plant per pot. As usual, 8" pots were used and filled with a sand-low P soil mixture and 200 mg of rock phosphate per kg of mixture. Plants receiving the low P treatment

appeared to be very stunted compared to those receiving the control treatment. Data analysis revealed large plant-to-plant variation within entries, little consistency across replications, and problems of plant establishment.

From there on, we decided to look back at the different trials that had been conducted over the past two years to establish this protocol. Careful examination of the previous experiments (2003-2004) conducted with the mapping population parents revealed several important factors: (i) the mean biomass response of low P plants varied a lot across experiments, reaching from as low as 5-10% of control to up to 40-50%, indicating probably that large variations occurred in the P levels of the different soil lots used in the experiments; (ii) earlier experiments carried out with urea tended to have larger biomass yield means than later experiments carried out with ammonium nitrate, showing that N source may have played an important interaction with the observed low P treatment responses (for understandable reasons); (iii) yet, there was still a good correlation between the biomass yield values obtained from the set of pearl millet mapping population parents throughout time.

After this exercise, we have then addressed four issues: (i) the differences in Olsen P in the different batches of soil, (ii) the control of plant to plant variation; (iii) the source of nitrogen; (iv) the possibility to favor initial plant establishment with early application of tiny doses of soluble P.

The issue raised in (i) was confirmed coincidentally when we decided to bypass the sand acid-washing step from our protocol to save time, resources, and avoid contamination. We ran an experiment to compare the biomass response of a set of 4 pearl millet genotypes grown in low P soil mixed (1:1 w/w) either with acid-washed sand or non acid washed sand. To our surprise, pearl millet genotypes grew about twice as well in the mixture using the acid washed sand as in the one with non-acid washed sand. Soil samples from the mixture had been kept and revealed that Olsen P was 0.9 and 1.5 ppm in the mixtures using non-acid washed and acid-washed sand, respectively. This experiment indicated that: (i) very slight differences in Olsen P triggered large differences in the plant response; (ii) plant to plant variation seen in other experiments might be due to minute differences in P availability, leading to differences in plant establishment (iii) in this experiment, plant establishment was good and subsequent plant to plant variations remained minimum.

Regarding issue (ii), we now measure soil and sand Olsen P each time a new batch is used, and we ensure that Olsen P of the soil mixture is above 1.5 ppm (lower values lead to severe problems of early plant establishment). Mixing is thoroughly done using a concrete mixer to ensure homogeneity of the soil mixture. In the low P field where soil is collected, we have also applied fertilizer P in one strip, which we can use as "inoculum" in case the Olsen P value of a batch of soil turns out too low. We also grow 3 plants per pot. Preliminary indications show that the problem of plant-to-plant variation is now under control.

Regarding issue (iii), we have carried out two separate experiments. Using a set of 4 hybrids, we have found that, indeed, urea-grown plants under low P grow significantly better than under ammonium nitrate. However, there is also a significant N × genotype interaction in a sense that one genotype grows as well under low P conditions regardless of whether N is provided from NH_4NO_3 or urea. In a second experiment, we grew the same set of genotypes in 4 different soils. Data are currently being analyzed. The visual differences were very obvious regarding the overall differences between NH_4NO_3 and urea. This means that the phenotypic screening of pearl millet mapping population progenies for differences in phosphorus acquisition ability, which is foreseen, will need to be done with both N sources.

Finally, regarding issue (iv), the possibility to add tiny amounts of P to the young seedlings was initially thought to decrease variation from plant to plant. In a preliminary experiment, we had the same 4 genotypes as above, grown in low P soil, and we treated plants with a) nothing, b) soaking one night in a 100mM KH_2PO_4 concentration, and c) applying 10 times the amount of P contained in an average pearl millet seed in a soluble form at 4 days after sowing. For plants receiving the +P treatments, we found that shoot biomass at 40 DAS was more than double of those receiving no P treatment, and this response varied across genotypes. A repeat of this experiment has been done using three soils, and three "priming" application of soluble P: 5, 10, and 20 times the amount of P in one seed. There again, we have found an important response to early application, with maximum effect being 10 times the amount in one seed. These results are very important and exciting, as they show that a very minor early application of P gives over a 40-fold return in P acquisition by the plants. Combined with issue (iii), urea plus initial "priming" give the base for a very good and applicable package.

Vadez V

Output 1.1.5	**Improved Nutritional Quality**
Activity 1.1.5.1	Marker-assisted genetic improvement of grain carotenoid content in pearl millet
Team	Hash CT, Rai KN, Chandra S
Milestones	Markers for grain carotenoid content mapped in pearl millet (2008)
	MAS for enhancement of grain carotenoid content in pearl millet initiated (2008)

Attempts to complete NIRS calibration for pearl millet grain carotenoid content following HPLC repairs were unsuccessful as correlation between two HPLC runs was not significant for the panel of 90 S1 and inbred progenies. No further work was undertaken in 2005 as required special project funding was not available, and no future work is projected unless special project funding is forthcoming.

Hash CT

Activity 1.1.5.2	Genetic engineering of groundnut for enhancement of β–carotene and of pigeonpea for enhancement of methionine and β–carotene
Team	Vanamala A, Sharma KK, Nigam SN, Saxena KB
Milestones	Transgenic groundnut lines with over-production of b-carotene evaluated under controlled field conditions (2007).
	Transgenic pigeonpea lines with over production of methionine and β–carotene evaluated under controlled field conditions (2007).

***Gene constructions of β–carotene into plant expression vectors*:** The in-house cloned genes encoding for phytoene synthases, *psy*1 from maize and *crt*B from *Erwinia herbicola*, involved in β–carotene biosynthesis were sequenced and the gene sequences were confirmed by alignment and blast analysis. The coding sequences of these genes were initially fused to constitutive expression promoter (CaMV 35S), sub-cloned into T-DNA region of the binary vector pCAMBIA 2300 and mobilized into *Agrobacterium tumefaciens* strain C 58 for genetic transformation studies. The expressions of the in house cloned genes were verified initially by transforming tobacco as a model system. Subsequently, the coding sequences of *psy1* or *crt*B genes were fused with the oleosin promoter to drive gene expression in the oil bodies of matured seeds. These gene constructs were

further sub-cloned into plant expression vector pCAMBIA 2300 and mobilized into *Agrobacterium* C 58 strain for genetic transformation of groundnut and pigeonpea. To generate marker-free transgenic plants, these gene constructs were also sub-cloned into binary vectors of pCAMBIA 2300 (minus *npt*II) and 2 T-DNA.

Construction of plant expression vectors containing sunflower seed albumin gene (SSA): The methionine-rich sunflower seed albumin gene (*SSA*) driven by the vicillin promoter (for seed specific expression), a 3.2 kb *Eco*RI fragment of pLT4 plasmid, was sub-cloned into binary plasmids pHS723, pCAMBIA 1301 and pCAMBIA 1302, and mobilized into *Agrobacterium* strain C 58 for genetic transformation of pigeonpea. Similarly, the same gene construct is being sub-cloned into pCAMBIA 2300:j*npt*II binary vector to produce marker-free transgenic plants.

Development and analysis of putative groundnut transgenic events carrying b-carotene genes: *Agrobacterium*-mediated genetic transformation of the selected genotypes of groundnut was carried out by using with newly constructed binary vectors containing β–carotene genes (*psy*1 and *crt*B) for obtaining high frequency of transformants. The putative transgenic groundnut shoots obtained under antibiotic selection pressure were elongated for rooting followed by their transfer to containment greenhouse.

The putative transgenic T0 groundnut plants growing in the containment greenhouse were analyzed molecularly for the integration and presence of the transgenes by using PCR with gene specific primers and Southern hybridization for the copy number. Initial molecular characterization from few putative transgenic groundnut plants showed the integration of *psy*1 gene. The transgene expression and the presence of m-RNA transcripts (*psy*1) were observed in few putative groundnut transgenic events by RT- PCR analysis and these events are being advanced for further generations.

Development of pigeonpea transgenic events for enhanced level methionine: The binary vector gene constructs containing *SSA* gene along with vicillin promoter (pHS723:SSA) in *Agrobacterium tumefaciens* strain C 58 is being regularly used for genetic transformation of pigeonpea for obtaining enhanced level of seed methionine content. In separate studies, *Agrobacterium*-mediated genetic transformation for selected genotypes of pigeonpea is being carried out regularly using with newly constructed binary vectors containing maize *psy*1 gene for generating transgenic events with enhanced level of β–carotene. The putative transgenic pigeonpea shoots obtained under antibiotic selection pressure are being elongated for rooting and transfer to the containment greenhouse for further analysis.

The primers specific to the coding sequence of *SSA* and *psy*1 genes were designed and conditions for PCR amplification optimized. The primary putative transformed pigeonpea shoots obtained under antibiotic selection pressure are being molecularly analyzed by PCR analysis for the presence of *SSA* and *psy*1 genes. The expression of *SSA* gene in transgenic tobacco was verified (used as a modal system) by RT-PCR analysis in the T1 seeds of tobacco.

Vanamala A, Sharma KK, Rai M, Nigam SN and Saxena KB

Output 1.1.6	**Improved feed and fodder quality**
Activity 1.1.6.1	Improved pearl millet stover quality
Team	Hash CT, Blummel B, Bidinger F, Folkertsma R, Mgonja M, Rattunde F, Chandra S

Milestone — Genotype and genotype × environment effects on stover quality documented, heritability of different stover quality components assessed, and sampling methods for efficient assessment of stover quality established in pearl millet (2006)

Management and genotype effects on pearl millet stover quantity and quality: Farmers have two basic options for improving both the quantity of stover produced and its nutritional quality - intensifying crop management to increase production and/or feed value of stover, and choice of cultivar type and/or specific cultivar to exploit genetic differences in quantity and/or quality of stover produced. We conducted a preliminary evaluation of both options for pearl millet, as baseline information for a funded project on the genetic improvement of millet stover quality.

The most significant management option available to farmers for increasing pearl millet stover digestible dry matter (DDM) yield and stover metabolizable energy (ME) yield is adequate fertilization. Although increased fertilizer application had small, but significant, negative effects on stover digestibility, sugar concentration and ME, these were more than offset by large increases in total stover dry matter production. Higher fertility also had a major positive effect on stover nitrogen concentration, which should have a significant effect on animal weight gain (especially where stover is fed without supplementation with either a concentrate or a higher N legume straw). Increasing plant population, in contrast, had little effect on either stover quality or yield. The low population treatment still produced nearly the same biomass and stover yields as the high population treatment, despite a two-fold difference in plant numbers. Although stover from the lower plant population treatment did have slightly numerically higher values for almost all stover quality traits, but differences were only significant for N % and digestibility, and these had little effect on stover DDM or ME productivity.

Choice of cultivar type (landrace, open-pollinated dual-purpose cultivar, or F_1 hybrid) did have a significant effect on both stover productivity and quality. The dual-purpose cultivars (and the landraces) had higher stover productivity and significantly higher digestibility, sugar concentration and ME than the hybrids, but this was at the cost of a significantly lower grain yield. The most important finding was that there were no strong negative relationships between the most important stover quality traits and either grain or stover yield, however, so there is not bar to combining high grain yields with high stover yields with at least average stover quality. Several dual-purpose hybrids in the trial (notably HHB 60 and the old ICRISAT release ICMH 451), capitalized on this, producing DDM and ME yields on par with the best open-pollinated dual-purpose cultivars and landraces, with a significantly higher grain yield. The results suggest that there is no reason why targeted breeding of dual-purpose hybrids (stover and grain yield) with improved stover quality should not be successful. Such hybrids should also maximize returns to investment in increased fertilization, though increased grain and quality stover yields.

Bidinger FR and Blümmel M

Milestone — Additional markers for key components of pearl millet stover quality (digestibility, N content, intake, etc.) identified (2006)

Linkage map construction and QTL mapping: New SSR, EST (EST-SSR and SSCP-SNP) and TRAP markers (developed by the University of Nebraska, USA; JIC, Norwich, UK; and ICRISAT-Patancheru, India) were added into the existing linkage map of the pearl millet mapping population based on cross ICMB 841 × 863B, which was comprised of the previously available polymorphic RFLP and SSR markers. Markers were assigned to different linkage groups based on marker

recombination and ordered within linkage groups to minimize the frequency of candidate errors. After removing colinear markers and those exhibiting distorted segregation patterns, seven linkage groups were constructed using 76 polymorphic RFLP, SSR and EST markers.

QTL analysis was performed by composite interval mapping for stover quality traits of individual stover fractions (stem, sheath, and leaf blade) as well as whole stover samples. Stems are a major and important constituent among the different stover fractions because they contribute more mass to the livestock feed. For stem fraction digestibility and metabolizable energy content, a major QTL was identified on LG 2, at a position similar to that for drought tolerance alleles from the 863B parent (suggesting it should be simple to combine improved stem digestibility with greater drought tolerance in the genetic background of elite seed parent maintainer line ICMB 841). Another major stover quality QTL was mapped to LG 5, which controlled significant proportions of phenotypic variation for *in vitro* true digestibility and for metabolizable energy content. In contrast, the ICMB 841 alleles for QTLs on LG 6 were favorable for gas volume, true organic matter degradability, nitrogen content and metabolizable energy content of stover stem and sheath fractions.

Nepolean T, Senthilvel S, Hash CT and Blümmel M

Milestone — Elite hybrid parental lines of pearl millet with improved stover quality through marker-assisted backcrossing available for evaluation (2007*)*

***Advancing QTL introgression lines*:** During the 2004/05 post-rainy season, 16 F_1 hybrids of selected hybrid seed parent maintainer lines (ICMB 95111, ICMB 95222, HMS 7B and ICMB 93333) and parents of two stover quality mapping populations (ICMB 841, 863B, PT 732B and P1449-2) were grown in the field. Each hybrid combination was backcrossed with the recurrent parent and resulting in a total of 16 F_1 parent × recurrent parent BC_1F_1 combinations. Two plant × plant crosses from each combination were selected to advance the BC_1F_1 generation. Two crosses from each of the BC_1F_1 hybrid combinations were sown and raised during the rainy season of 2005. BC_1F_1 hybrids were backcrossed, plant × plant, with their respective recurrent parents to produce BC_2F_1 seeds. The BC_1F_1 generations of 16 crosses were screened using polymorphic SSR and TRAP markers, to assess their segregation patterns and select the heterozygous QTL introgression genotypes from various crosses for generation advance.

For the putative LG 7 stover quality QTL, RFLP and SSR marker data were used to identify introgression homozygotes for the associated region from donor parent 863B in the genetic background of ICMB 841. Several such plants were identified and their selfed seed harvested for future testcross hybrid production.

The revised QTL analysis reported above indicated that at least some fo the introgression lines for the LG 2 drought tolerance QTL of 863B in the genetic background of ICMB 841 are likely to carry a QTL for improved stover quality.

Nepolean T and Hash CT

Milestone — Two cycles of recurrent selection for improved stover quality (using NIRS analysis) completed in two arid zone landrace-based populations in pearl millet (2007*)*

Evaluation of ICMV 221 cycle 2 FS (full-sib) progenies: We completed the second and final cycle of FS progeny selection for improved stover quality in the released pearl millet variety ICMV 221.

The objective of the exercise is to measure the genetic progress that can be made in improving the ruminant nutritional quality of pearl millet stover using simple recurrent selection methodology and rapid near-infrared reflectance spectroscopy (NIRS) assessment of key stover quality parameters. As in the first cycle, there were highly significant differences for all stover quality traits and agronomic variables measured, among the 280 FS progeny evaluated. For example stover *in vitro* organic matter digestibility ranged from 37 to 46%, stover metabolizable energy from 5.2 to 6.7 mega joule kg^{-1}, stover nitrogen from 0.67 to 1.25 %, and stover yield from 123 to 432 g m^{-2}. Selected progenies will be recombined to form both grain and dual-purpose versions of the original variety with improved stover nutritional quality, to compare to the original ICMV 221. Individual progenies will also be used to develop high stover quality trait lines for various research purposes, including evaluating the effect of improving parental stover quality on hybrid stover quality.

Bidinger FR and Blümmel M

Milestones

Stover quality of experimental pearl millet hybrids of BC_5 products evaluated in multilocational field trials (2008)

Stover quality of experimental varieties from selected arid zone populations in multilocational field trials evaluated in pearl millet (2008)

Response to a single cycle of selection for stover yield and quality: *We* random-mated selected progenies from the first cycle of improvement of the stover quality of the pearl millet variety ICMV 221 (see above) to produce stover quality trait-based experimental varieties to assess the potential for reselecting an existing variety to improve its stover quality. Selection was done jointly for a high or low value of the quality trait, plus grain and stover yield within one SED for mean of the whole set of progenies. In addition we made specific grain or dual purpose versions of the original variety with as good a stover quality as was possible within a high grain or grain + stover yield objective. All six experimental varieties and the C0 and C1 versions of the original ICMV 221 were tested in replicated small plots for productivity and *in vitro* quality analysis by NIRS, and a subset was grown in replicate large plots for *in vivo* quality analysis in a replicated sheep feeding trial.

The reselected grain type experimental variety did not differ significantly from the parent variety, either in agronomic performance (apart from some offsetting changes in yield components) or in stover quality in either the *in vitro* or *in vivo* measurements. The dual propose experimental variety, in contrast, exceeded the parent variety in stover yield by 12% ($P < 0.05$) and in grain yield by 5% (NS). More interestingly, although it did not differ from the parent variety in the *in vitro* quality evaluations, it was significantly better than the parent for organic matter digestibility (56.6% vs. 54.4%) in the *in vivo* evaluation.

Selection for high and low stover digestibility and for high stover nitrogen generally resulted in small and non-significant reductions in both grain and stover yield. The exception was the experimental variety made on the basis of low stover nitrogen, which resulted in a 6% ($P<0.05$) increase in biomass and a 10% ($P<0.05$) increase in grain yield. Selection for and against stover digestibility and stover N% resulted in small, but non-significant, changes in these parameters, in the direction of selection in the *in vitro* stover quality assessment, but few other changes in quality. In the *in vivo* assessment however, the high digestibility selection had a significantly higher digestibility (57.5%) than the parent variety (54.5%).

Bidinger FR and Blümmel M

Global Project 2

Applications of biotechnology for improving host plant resistance to insect pests and diseases

[Project Coordinator: HC Sharma]

Output 1.2.1	**Marker-assisted selection for enhanced resistance to insect pests and diseases**
Activity 1.2.1.1	Mapping stem borer/midge resistance in sorghum
Team	Sharma HC, Hash CT, Reddy BVS
Milestone	Mapping population evaluated for resistance to stem borer

***Reciprocal crosses of RILs for stem borer resistance [ICSV 745 × PB 15520-1 and ICSV 745 × PB 15881-3] advanced*:** During the 2004 *rabi* season, a total of 935 $F_{3:9}$ progenies of stem borer RILs and their bulks were advanced to $F_{3:10}$: 272 $F_{3:9}$ of ICSV 745 (stem borer-susceptible, but midge-resistant and agronomically elite line) × PB 15520 (stem borer-resistant, but midge-susceptible and agronomically elite line), 300 $F_{3:9}$ of PB 15520 × ICSV 745, and 363 $F_{3:9}$ of ICSV 745 × PB 15881-3 (stem borer-resistant, but midge-susceptible and agronomically elite line).

Reddy BVS

***Evaluation of mapping population for resistance to spotted stem borer,* Chilo partellus:** Mapping population based on 296B ×IS 18551 (270 lines) was evaluated for resistance to spotted stem borer, *Chilo partellus*, under artificial infestation in a balanced design with three replications. Data were recorded on leaf feeding, deadheart formation, leaf glossiness, days to panicle initiation, recovery resistance, and agronomic score. Leaf damage rating (DR) varied from 4.0 – 8.0 in the mapping population (1 = <10% leaf area damaged, and 9 = >80% leaf area damaged) as compared to 5.5 in IS 2205 – the resistant check, and 8.5 in ICSV 1 - the susceptible check. IS 18551 suffered a DR of 6.5, while 296B suffered a DR of 8.0. Deadheart formation ranged from 5.6 to 90.3% in the mapping population, 24.1% in the resistant check - IS 2205, and 63.0% in the susceptible check, ICSV 1. The susceptible parent 296B showed 71.3% deadheart formation compared to 25.5% in the resistant parent – IS 18551. Leaf glossiness score varied from 1.0 – 5.0 in the mapping population (1 = highly glossy, and 5 = non-glossy) as compared to 1.0 in IS 2205 – the resistant check, and 5.0 in the susceptible check - ICSV 1. Leaf glossiness score was 5.0 in 296B and 1.0 in IS 18551.

The mapping population, ICSV 745 × PB 15881-3, was evaluated for resistance to sorghum midge, *Stenodiplosis sorghicola* during the 2004/05 post-rainy season. There were three replications in a balanced randomized complete block design (RCBD). Data were recorded on midge damage on a 1 to 9 rating scale (1= <10% spikelets with midge damage, and 9 = >80% spikelets with midge damage). Midge damage in the mapping population ranged from 2.0 to 9.0 compared to 1.0 in the resistance check, ICSV 197, and 7.7 in the susceptible check, Swarna. The resistant (ICSV 745) and the susceptible parents (PB 15881-3) suffered a midge DR of 2.0 and 6.3, respectively.

Sharma HC, Reddy BVS and Hash CT

Activity 1.2.1.2	Mapping shoot-fly resistance in sorghum
Team	Hash CT, Reddy BVS, Sharma HC, Chandra S
Milestone	RILs and backcross populations advanced to facilitate marker-assisted selection for shoot fly resistance (2008)

Marker-assisted selection for resistance to shoot fly, Atherigona soccata in sorghum. Sorghum RILs [252 RILs from the cross 296B (susceptible) × IS 18551 (resistant)] were phenotyped and genotyped to understand the genetics of shoot fly resistance and locate chromosomal regions harboring putative quantitative trait loci (QTLs) for shoot fly resistance and related component traits [PhD thesis, "QTL analysis for shoot fly resistance in sorghum [*Sorghum bicolor* (L.) Moench", by SP Deshpande, submitted in 2005 to Marathwada Agricultural University (MAU)-Parbhani, Maharashtra, India]. After genotyping the RIL population with SSR markers, phenotypic observations of off-type progenies were confirmed. As a result, the effective size of the mapping population was reduced to 213 RILs, for which phenotypic screening data (from two field screens for shoot fly resistance conducted at MAU-Parbhani) could be combined with the marker data set (111 SSR loci) for linkage analysis and QTL mapping.

The linkage map for this RIL population covers 2165.8 cM (Haldane units), providing at least partial coverage of all ten sorghum linkage groups, but regions with poor marker coverage remain. Composite interval mapping revealed the presence of putative QTLs for all important shoot fly resistance traits, accounting for 6 to 36% of observed phenotypic variances for these traits. One major QTL for glossiness was detected on LG J, accounting for 33% of observed phenotypic variation. Minor QTLs for seedling vigor, deadhearts , and seedling height were also detected. Co-localization of a QTL for trichome density on the upper leaf surface (explaining 20% of observed phenotypic variation) with a QTL for trichome density on the lower leaf surface (explaining 25% of observed phenotypic variation) indicated similarities in genetic control of trichome densities on either side of sorghum leaf blades. The results obtained largely confirmed the previous information based on the BTx623 × IS 18551 mapping population. It would be desirable to fill some of the remaining gaps in the linkage map, if appropriate markers can be identified, and repeat the QTL analyses with all available shoot fly resistance screening data sets for the 213-entry subset of the (296B × IS 18551)-based sorghum RIL population.

QTL mapping of shoot fly resistance, based on two phenotypic screens conducted at ICRISAT-Patancheru of the sorghum RIL mapping population based on the cross 296B × IS 18551, which had been genotyped previously, was also undertaken [PhD thesis, "Genetic diversity analysis, QTL mapping and marker-assisted selection for shoot fly resistance in sorghum [*Sorghum bicolor* (L.) Moench" by SP Mehtre, submitted in 2006 to MAU, Maharashtra, Parbhani, India] . Analysis of the phenotypic data was performed using the residual maximum likelihood algorithum (ReML), which provides the best linear unbiased predictors (BLUPs) of performance of the genotypes. The BLUPs of 213 uniform RILs, along with their genotypic data from 111 marker loci, were used for QTL analysis. Parental and RIL BLUPs revealed wide variation in phenotypic values for shoot fly resistance and its component traits in each of the screening environments. Wide variation was observed in the RIL population for shoot fly resistance component traits such as leaf glossiness, trichome density (upper and lower surfaces of seedling leaf blades), seedling vigor, oviposition preference, and deadhearts. These traits can be used as simple criteria to select for resistance to shoot fly in sorghum. The genotypic variances for shoot fly resistance traits were significant in each of the screening environments as well as across-environments. Glossiness intensity, trichome density (both upper and lower surfaces of seedling leaf blades), oviposition preference, deadhearts, and seedling vigor showed consistent heritability (broad-sense) estimates in individual screening environments, but low to moderate heritability estimates across environments,indicating that these traits are under genetic control, but there is a substantial role of genotype (G) × environment (E) interaction in expression of these traits.

QTL analysis was performed using the composite interval mapping (CIM) method implemented in PLABQTL version 1.1, which uses a regression approach. This revealed presence of putative QTLs for shoot fly resistance and its component traits including leaf glossiness, oviposition preference, deadhearts, and trichome density. The portion of observed phenotypic variance explained by different putative QTLs varied from 6 to 34%. Glossiness intensity was largely controlled by a major QTL on LG J (= SBI-05), accounting for 34% of observed phenotypic variation, and one minor QTL on LG G (= SBI-10), accounting for 8% of observed phenotypic variation across environments. After adjusting for QTL × environmental interaction, these two QTLs explained 31% of genetic variation in glossiness intensity in this population. Resistant parent IS 18551 contributed to additive genetic effects for increased glossiness at both of these QTLs. For oviposition preference and deadhearts, two common QTLs (one on LG F = SBI-09 and one on LG G) were also mapped in across-environments analysis. Together, these QTLs explained 17% phenotypic variation in oviposition preference and 19% for deadhearts in across-environments analysis. Significant QTL × environmental interactions were observed for these QTLs for oviposition preference and deadhearts. The QTL mapped on LG G for deadhearts and oviposition preference co-localized with a major QTL for trichome density (upper and lower surfaces of seedling leaf blades) and a minor QTL for glossiness intensity. The QTL mapped on LG F for deadhearts and oviposition preference co-localized with a minor QTL for trichome density on the lower leaf surface. For trichome density on the upper leaf surface, one QTL was detected on LG G accounting for 30% of observed phenotypic variance in across-environments analysis. This QTL co-localized with a QTL for trichome density on the lower leaf surface, and explained nearly 27% of observed phenotypic variance across two screening environments. The major QTL for glossiness intensity and a minor QTL for oviposition preference (LG J = SBI-05) and a major QTL for trichome density and minor QTLs for glossiness, deadhearts, and oviposition (LG G = SBI-10) detected in this study have previously been mapped at the same location in another sorghum RIL population derived from the cross BTx623 × IS 18551. This confirmed that these chromosomal regions might be harboring candidate genes contributing to shoot fly resistance in IS 18551.

During 2005, SSR-marker-assisted backcrossing of putative shoot fly resistance QTLs from donor parent IS 18551 into the genetic backgrounds of three hybrid parental lines (20B, 28B, and KR 192) of interest to the sorghum breeding program at the Marathwada Agricultural University-Parbhani, Maharashtra, India, were advanced to the BC_3F_1 seed generation. For this purpose, around 224 BC_2F_1 plants from five populations were genotyped at the seedling stage with 10 SSR marker loci linked to four targeted QTLs (one each on LG A = SBI-01, LG E = SBI-07, LG G = SBI-10, and LG J = SBI-05) associated with shoot fly resistance traits. Nearly 100 heterozygous plants, which had one, two, or more QTL introgression(s), were selected and crossed as a female parent with the selfed progeny of their respective recurrent parents to generate BC_3F_1 progenies.

SSR-marker-assisted backcrossing of putative shoot fly resistance QTLs into the genetic background of the susceptible parents of the two ICRISAT sorghum shoot fly mapping populations, i.e., BTx623 and 296B, were advanced during 2005. By the end of 2005, three of the four target QTLs from donor parent IS 18551 were advanced to the BC_4F_2 seed generation in the genetic background of shoot fly susceptible elite sorghum hybrid seed parental line 296B, and all four QTLs were advanced to the BC_4F_2 seed generation in the genetic background of shoot fly susceptible elite sorghum hybrid seed parental line BTx623. It is expected that the first BC_4F_2 shoot fly resistance QTL introgression homozygotes will be identified by mid-2006.

Folkertsma RT, Reddy BVS, Sharma HC, Hash CT, Chandra S and Senthilvel S

Activity 1.2.1.3 Mapping head bug/grain mold resistance in sorghum

Team Thakur RP, Hash CT, Sharma HC, Reddy BVS, Rattunde EW

***RILs developed for grain mold resistance*:** Two grain mold RIL populations; 352 $F_{3:4}$ progenies of IS 23599 (grain mold resistant line) × AKMS 14B (grain mold susceptible line), and 348 $F_{3:4}$ progenies of IS 25017 (grain mold resistant line) × KR 188 (grain mold susceptible line), were advanced to $F_{3:5}$ progenies in the 2004/05 *rabi* season. Limited information available so far indicates that SSR marker polymorphism between parental line pairs of these populations may not be adequate to permit QTL mapping with sorghum SSR markers currently available in the public domain. Assessment of a sample of the RILs for variation in plant height and flowering time, as well as panicle compactness, in an appropriate grain mold screening environment is recommended as previously published attempts in the USA to map QTLs for the related grain weathering complex were largely ineffective as all "grain mold resistance" QTLs identified were either associated with alleles for tall plant height at dwarfing gene loci (that contributed to drier microenvironments for panicles borne by taller progenies in the RIL population) or with grain hardness QTLs.

Reddy BVS, Ramesh S, Folkertsma RT, Hash CT and Thakur RP

Activity 1.2.1.4 Mapping *Striga* resistance in sorghum

Team Folkertsma R, Hash CT, Rattunde EW

Milestone EST-derived markers closely linked to *Striga* resistance in sorghum identified (2006)

Sequencing of gene-rich regions of the sorghum genome has moved ahead rapidly, as evidenced by the November 2004 release by Orion Genomics to the public domain of over 500K methyl-filtered sequence reads, predicted to include at least a portion of the sequence of 95% of all sorghum genes (more than 784K sorghum DNA sequences are now available in GenBank). Interestingly, some 25K of the new methyl-filtered sequence reads contain SSR repeat motifs. Thus, the currently available sorghum DNA sequence information would allow substantial expansion of sequence-tagged microsatellite (STMS) marker resources for sorghum, and could provide polymorphic co-dominant PCR-compatible markers in large numbers across the entire sorghum genome. Even more interesting is that by applying simple, inexpensive bioinformatics protocols, we can identify with reasonable certainty where many of these new STMS markers have counterparts in the rice genome sequence, and hence can predict where they will map on the sorghum genome.

Using this approach, (as part of the PhD thesis research program of Mr. P. Ramu), sorghum EST sequences from The Institute for Genome Research (TIGR) database were searched for the presence of SSRs in their sequences. The non-redundant sorghum EST sequences with SSR motifs were searched against the rice genome sequence in the Gramene database (using BLAST). Among the hits identified, those having the highest score were checked for its location on the rice genome. Nearly 2,000 non-redundant sorghum EST sequences containing SSRs were searched against the rice genome. For each rice linkage group, 50 hits with map positions distributed across the full length of the linkage group were selected. This revealed rice chromosomal locations of similar sequences, allowing selection of a subset of 600 sorghum EST-SSR loci distributed across regions of the sorghum genome that are syntenic with each of the 12 rice chromosomes (50 each). These sorghum EST-SSR loci, if polymorphic in sorghum, are expected to provide coverage across the entire nuclear genome of sorghum. Primer pairs flanking the repeat sequences in each of these 600 sorghum EST sequences were then designed (after masking repeat regions) using the Primer3 program (www.genome.wi.mit.edu). PCR optimization and polymorphism assessment of primer pairs for

these 600 candidate sorghum EST-SSR markers were taken up simultaneously using as template DNA samples from the parental lines of several ICRISAT sorghum RIL populations, including those previously used for mapping *Striga* resistance.

During 2005, a subset of the E36-1 × N 13-based RIL population has been used (by PhD student, Kassahun Bantte) to start mapping some of the polymorphic EST-SSR markers. Genotyping has been completed and mapping initiated for about 50 EST-SSR loci expected to map to four of the ten sorghum linkage groups (SBI-01, SBI-02, SBI-03, and SBI-05). Based on the marker data generated, 44 of these EST-SSR markers were mapped across all 10 sorghum linkage groups (not just those actually targeted). Among these 44 newly mapped markers, four were closely linked to the *stg*B QTL on LG B = SBI-02 (which was a primary target of this exploratory mapping exercise). These findings will facilitate future development of additional sorghum EST-SSR markers specifically targeting QTLs for any mapped trait, including *Striga* resistance, for use in marker-assisted selection. With completion of this activity, if we need more SSR markers in a particular region of the sorghum genome, we hope to be in a position to quickly and inexpensively develop them, using the vast amount of sorghum sequence information that is rapidly becoming available.

Folkertsma RT, Hash CT, Jayashree B and Haussmann BIG

Striga resistance in sorghum transferred to elite African cultivars using marker-assisted selection. Marker-assisted backcrossing of *Striga* resistance QTLs from donor parent N 13 into the genetic background of farmer-preferred sorghum varieties from Mali, Sudan, Kenya, and Eritrea were advanced more slowly than planned during 2005. Logistical problems in moving DNA samples, and/or appropriate tissue samples for DNA isolation between national sorghum breeding program sites (where the crossing and backcrossing activities are undertaken) and the BecA facility in Nairobi, Kenya (where the SSR marker genotyping activities are undertaken), are hampering progress towards this milestone.

Folkertsma FR, deVilliers S, Hoisington D, Kiambi D and Hash CT

Arresting the scourge of Striga in sorghum in Africa by combining the strength of marker-assisted backcrossing with farmer-participatory selection. Through this project, NARS in the Kenya, Mali, Eritrea and Sudan are being assisted to strengthen *Striga* resistance of farmer-preferred sorghum varieties (FPSVs) through a combination of marker-assisted backcrossing (MAB) and farmer-participatory selection. The stability of inheritance of the transferred *Striga* resistance alleles in the FPSVs, the actual out-crossing rates in selected FPSVs, and the pollen flow of these FPSVs is being analyzed in order to develop recommendations for variety maintenance and on-farm seed production. To complement the molecular work, a socio-economic and population genetics study of the sorghum seed supply systems in the four target countries is being undertaken concurrently to guide the design of effective seed interventions by partner institutions so that improved materials efficiently reach farmers.

The first generation of backcrosses between F_1 and the farmer-preferred varieties (Hugurtay, Hiryray, Ochuti, Tabat, Wad Ahamat, Tiemarifign, and CSM 335) has been performed and BC_1F_1 generated in Kenya, Mali, Sudan, and Eritrea. In Kenya, 210 BC_1F_1 individuals were genotyped using 3 SSR markers: 63 plants were found to be heterozygous for markers in the QTL target regions – 3 plants contained 3 *Striga* QTLs, 13 had 2 *Striga* QTLs, and 47 had 1 *Striga* QTL. Confirmed hybrids were backcrossed to the FPSV recurrent parent Ochuti to produce the BC_1F_1 generation, which is currently being genotyped. In Sudan, 144 Tabat BC_1F_1 samples were genotyped and 28 plants were found to be heterozygous for markers in the QTL target regions – 5 plants contained 4 *Striga* QTLs,

4 contained alleles of 3 QTLs, 5 had 2 QTLs, and 12 had 1 QTL. Capacity for DNA isolation, quantification, PCR optimization, and agarose/PAGE with silver staining has been provided to the NARS in Mali (UB and IER), and Kenya (KARI-Katumani) through training, provision of equipment, and technical backstopping.

A gene flow experiment was set up to determine the distance of pollen flow using male-sterile lines as receptors, thus eliminating the need of large scale PCR genotyping. The flowering dates of the materials from Sudan, Kenya, and the male-sterile lines were determined and the first pollen dispersal experiment carried out using Ochuti and N 13 pollen donor and recipient, respectively. The experiment is currently being repeated at another site. A field study has been conducted on sorghum indigenous knowledge, farming system, stakeholders' perceptions and seed supply systems in Eritrea, Kenya, and Sudan. A report containing the findings of status of the seed sub-sector in Eritrea, Kenya, and Sudan has been compiled.

Kiambi D

Activity 1.2.1.5.	Mapping downy mildew resistance in pearl millet
Team	Hash CT, Thakur RP
Milestone	Cultivars derived through MAS for downy mildew resistance released for cultivation to the farmers (2008)

First product of pearl millet marker-assisted selection, "HHB 67 Improved", eleased for cultivation in India. The year 2005 saw the first official release for seed multiplication and cultivation of a product of marker-assisted selection "HHB 67 Improved" for downy mildew resistance in pearl millet. This pearl millet hybrid was identified for state release in the Indian state of Haryana in January 2005, and was later identified (in July) at the national level for cultivation in the arid zone of northwestern India, where extra-early maturing pearl millet hybrid "HHB 67" has been cultivated on nearly 500,000 ha annually.

"HHB 67 Improved" is the first product of marker-assisted selection, other than transgenic Bt cotton hybrids, to be approved for release in India. The new hybrid was bred by backcrossing additional downy mildew resistance into the seed parents (using conventional greenhouse seedling screening procedures and resistance donor ICML 22) and the pollinator parent (using RFLP-based marker-assisted selection and resistance donor ICMP 451-P6) of "HHB 67", identifying BC_4F_3 families homozygous for resistance, and then performing line × tester experiments to identify agronomically superior hybrid combinations similar to "HHB 67", but having improved downy mildew resistance. The superior combinations were then tested multilocationally in on-station collaborative, state and national trials (2001-2004) with a smaller number being tested extensively in on-farm trials in Haryana (2003-2004), to identify the hybrid combination that has ultimately been released for cultivation as a high-yielding and disease-resistant replacement for "HHB 67".

Large quantities of breeder seed of the parental lines of "HHB 67 Improved" were distributed in 2005 to public and private seed agencies by ICRISAT and Haryana Agricultural University following approval of the hybrid's release by the central government in India. Due to regulatory delays in the release process, much of the breeder seed is being used for certified seed production of the new hybrid, rather than multiplication of foundation seed of the parental lines, that is normally used for producing certified seed. However, this should ensure that certified seed is available to sow >50,000 ha with "HHB 67 Improved" during the 2006 rainy season.

Hash CT and Thakur RP

Greenhouse screening for resistance to downy mildew: A total of 1443 lines, including inbreds, hybrids and self bulks of different populations were evaluated against three pathotypes (Sg 139 from Jodhpur, Sg 409 from Patancheru, and Sg 298 from New Delhi) of downy mildew pathogen, *Sclerospora graminicola* in 12 experiments. All screenings were carriedout in a completely randomized design having 3 replications, with 1 to 2 pots per replication, and 35 to 40 seedlings per pot. In each set, appropriate resistant and susceptible controls were included. In most of the sets, the common susceptible check 7042S recorded 95 to 100% disease incidence, indicating high effectiveness of the screening technique. The results of screening with frequencies of lines falling into different downy mildew incidence classes are presented in Table 2.1**,** and brief descriptions of material screened are given below. Lines showing ≤10% incidence were considered resistant.

Hybrids: A total of 53 hybrids made on 841B-like drought tolerance QTL introgression lines based on donor 863B-P2 were evaluated against three pathotypes (Sg 139 from Jodhpur, Sg 409 from Patancheru, and Sg 298 from New Delhi). Of these, 17 hybrids were resistant to Jodhpur pathotype, 5 to Patancheru pathotype, and 32 to New Delhi pathotype (Table 2.1). As it was considered useful to evaluate the performance of the resistant hybrids in their respective adaptation zones for further selection, the entire set of hybrids was also evaluated for agronomic performance in field trials at Central Arid Zone Research Institute (CAZRI), Jodhpur, Rajasthan, India, and Rajasthan Agricultural University, Regional Research Station (RAU-RRS), Nagaur, Rajasthan, India, during the rainy seasons of 2004 and 2005.

841B-like inbred lines: Fifteen 841B-like drought tolerance QTL introgression lines based on donor 863B-P2 were evaluated against three pathotypes (Sg 139 from Jodhpur, Sg 409 from Patancheru, and Sg 298 from New Delhi). Only one line each was resistant to the Jodhpur and Patancheru pathotypes, while 2 were resistant to the New Delhi pathotype. 863B remained highly resistant to all the three pathotypes while the remaining lines were susceptible. This indicated that 841B is now susceptible to this set of downy mildew pathotypes, which are broadly representative of the most virulent field isolates in India, and that the major downy mildew resistance QTL(s) from 863B are still effective against these pathotypes, and are not linked to the putative drought tolerance QTL on linkage group 2 of 863B. Pyramiding of downy mildew resistance and drought tolerance QTLs in the background of 841B will be necessary before an applied product can come out of the drought tolerance backcrossing program.

843B-like inbred lines: A total of 44 lines in the common background of 843B, but derived from backcrossing with downy mildew resistance donors ICML 22, P 7-3, and ICMP 85410 were evaluated against Patancheru (Sg 409), Jodhpur (Sg 139), and New Delhi (Sg 298) pathotypes. Only 4, 14, and 10 lines were resistant to pathotypes Sg 409, Sg 139, and Sg 298, respectively.

H 77/833-2-like inbred lines: Of 30 lines with a background of H 77/833-2 derived from downy mildew resistance donor ICMP 451-P6 and drought tolerance donor PRLT 2/89-33 evaluated against Patancheru (Sg 409), Jodhpur (Sg 139) and New Delhi (Sg 292) pathotypes, none showed resistance to Jodhpur (Sg 139) pathotype, while only 1 and 4 lines were resistant to Patancheru and New Delhi pathotypes, respectively. This indicated that H 77/833-2, ICMP 451-P6, and PRLT 2/89-33 are susceptible to these pathotypes, which are broadly representative of the most virulent field isolates in India; and pyramiding of downy mildew resistance and drought tolerance QTLs in the background of H 77/833-2 will be necessary before an applied product can come out of the drought tolerance backcrossing program. Crosses pyramiding the available drought tolerance QTL with downy mildew resistance QTLs in the background of H 77/833-2 were advanced by one generation.

Table 2.1: Summary of 2005 phenotyping of pearl millet mapping population progenies and products of conventional and marker-assisted backcrossing for resistance to different pathotypes (*Pat = Patancheru, Jdp = Jodhpur, Ndl = New Delhi) of pearl millet downy mildew (Sclerospora graminicola) (ICRISAT, Patancheru, India).

			Number of lines in each DM incidence class					
Material	**Pathotype***	No of lines	0 (%)	1-5 (%)	6-10 (%)	11-20 (%)	21-30 (%)	>30 (%)
Greenhouse screening								
Hybrids of 841B-like drought	Jdp (Sg 139)	53	6	5	6	16	13	7
tolerance QTL introgression	Pat (Sg 409)	53	4	0	1	10	9	29
lines based on donor 863B-P2	Ndl (Sg 298)	53	6	17	9	15	5	1
B-lines from 841B-like drought	Jdp (Sg 139)	15	1	0	0	0	0	14
tolerance QTL introgression	Pat (Sg 409)	15	1	0	0	0	0	14
backcrossing based on donor 863B-P2	Ndl (Sg 298)	15	1	0	1	0	4	9
843B-like inbred lines	Pat (Sg 409)	44	0	3	1	2	2	36
	Jdp (Sg 139)	44	5	7	2	0	0	30
	Ndl (Sg 298)	44	0	2	8	1	1	32
H 77/833-2-like inbred lines	Pat (Sg 409)	30	0	0	1	1	2	26
	Jdp (Sg 139)	30	0	0	0	0	0	30
	Ndl (Sg 298)	30	1	0	3	2	1	23
ACIAR pearl millet stover	Pat (Sg 409)	10	0	0	1	3	2	4
quality project trial	Jdp (Sg 139)	10	0	1	2	3	0	4
	Ndl (Sg 298)	10	0	2	2	1	2	3
BC_1F_2/BC_2F_1 progenies from (81B × IP 18293) × 81B	Ndl (Sg 298)	40	0	0	3	3	10	24
BC_1F_2/BC_2F_1 progenies from (843B × IP 18293) × 843B	Ndl (Sg 298)	52	0	0	0	4	7	41
BC_2F_2/BC_3F_1 progenies from (PT 732B × IP 1449-2) × 81B	Ndl (Sg 298)	169	0	0	0	0	4	165
BC_2F_2/BC_3F_1 progenies from (PT 732B × IP 1449-2) × 843B	Ndl (Sg 298)	389	0	0	0	5	16	368
BC_3F_2/BC_4F_1 progenies from (PT 732B × IP 1449-2) × PT 732B	Ndl (Sg 298)	144	8	5	15	21	16	79
F_4 self bulks (PT 732B-P2 × IP 1449-2-P1)	Pat (Sg 409)	131	0	0	0	0	0	131
F_4 self bulks (841B-P3 × 863B-P2)	Pat (Sg 409)	160	0	2	14	17	14	113
F_4 self bulks (89111B-P6 × 90111B-P6)	Pat (Sg 409)	206	0	10	7	17	15	157
Field Screening								
841B-like inbred lines		15	1	11	2	0	0	1
843B-like inbreds lines		44	0	6	6	7	8	17
H 77/833-2-like inbred lines		30	0	1	1	7	6	15
ACIAR pearl millet stover quality project trial		10	0	4	2	2	2	0

Improvement of stover quality: A total of 61 breeding lines being used for improving the stover quality of elite pearl millet seed parent maintainer lines were evaluated against Jamnagar (Sg 200), Jodhpur (Sg 139), Durgapura (Sg 212), and Patancheru (Sg 409) pathotypes. Only 2 lines (863B-P2 and ICMB 99022) were disease free, 18 were resistant to all three pathotypes, and 9 were resistant to 2 pathotypes. The results indicated that the elite seed parent maintainer lines selected for stover quality improvement also need to be improved for downy mildew resistance. As 863B-P2 is being used as one of the stover quality donor parents, and it is also highly resistant to the full range of pathotypes screened, it should be possible to select more downy mildew resistant derivatives from the segregating progenies using either conventional selection with seedling greenhouse screens of potted seedlings or RFLP/SSR/morphological marker-assisted selection.

Segregating breeding populations

***BC_2F_1s (81B × IP 18293) × 81B*:** Of 40 $B_{C1}F_2/BC_2F_1$ progenies from [(81B × IP 18293) × 81B] evaluated against New Delhi pathotype (Sg 298), only 3 progenies were resistant (≤10% incidence) compared to 45 and 89% incidence on IP 18293 and 81B, respectively. It seems that the resistance donor IP 18293 is now susceptible to the New Delhi pathotype, and needs to be confirmed. Disease incidence in the segregating progenies ranged from 6 to 97%, with an operational heritability >0.90. Segregation patterns permitted identification of BC_2F_1 progenies apparently carrying one or more partially effective resistance genes from donor IP 18293. Disease-free seedlings from eight BC_2F_1 progenies in four families were transplanted to the field and advanced a generation by selfing and backcrossing to produce families of BC_2F_2/BC_3F_1 progeny pairs, each expected to segregate for one or more partially effective resistance genes from IP 19283 in the background of 81B. This map-directed conventional backcrossing program is expected to produce near-isogenic lines carrying single additional downy mildew resistance genes from IP 18293 in the genetic background of 81B.

***BC_2F_1s (843B × IP 18293) × 843B*:** Of 52 BC_1F_2/BC_2F_1 progenies from [(843B × IP 18293) × 843B] × 843B evaluated against New Delhi pathotype (Sg 298), none of the progenies were resistant (and none was expected), while IP 18293 recorded 45 and 843B 98% disease incidence, respectively. Disease incidence in the segregating progenies ranged from 10 to 99%, with an operational heritability of >0.90. Segregation patterns permitted identification of BC_2F_1 progenies apparently carrying one or more partially effective resistance genes from donor IP 18293. Disease-free seedlings from six BC_2F_1 progenies in four families were transplanted in the field and advanced a generation by selfing and backcrossing to produce families of BC_2F_2/BC_3F_1 progeny pairs, each expected to segregate for one or more partially effective resistance genes from IP 19283 in the background of 843B. This map-directed conventional backcrossing program is expected to produce near-isogenic lines carrying single additional downy mildew resistance genes from IP 18293 in the genetic background of 843B.

***BC_2F_2/BC_3F_1s (PT 732B × P 1449-2) × 81B*:** Of 169 BC_2F_2/BC_3F_1 progenies from [(PT 732B × P 1449-2) × 81B] evaluated against New Delhi pathotype (Sg 298), none of the progenies was uniformly resistant (and none was expected). Downy mildew incidence levels of 29, 79, and 100% were recorded on the resistance donor P 1449-2, and elite seed parents 81B and PT 732B, respectively. Disease incidence in the segregating progenies ranged from 30 to 100%, with an operational heritability >0.90. Segregation patterns permitted identification of BC_3F_1 progenies apparently carrying one partially effective resistance gene from donor P 1449-2. A small subset of the BC_3F_1 progenies (seven progenies distributed across five of the six families screened) was selected for re-screening against Sg 298 in early 2006. Disease-free seedlings from one or more of the selected progenies in these families will then be transplanted in the field and advanced a generation by selfing

and backcrossing to produce families of BC_3F_2/BC_4F_1 progeny pairs, each expected to segregate for one or more partially effective resistance genes from P 1449-2 in the background of 81B. This map-directed conventional backcrossing program is expected to produce near-isogenic lines carrying single additional downy mildew resistance genes from P 1449-2 in the genetic background of 81B.

BC_2F2/BC_3F_1s (PT 732B × P 1449-2) × 843B: Of 389 BC_2F_2/BC_3F_1 progenies from (PT 732B × P 1449-2) × 843B evaluated against New Delhi pathotype (Sg 298), no individual segregating progeny was found uniformly resistant (and none was expected). Downy mildew incidence of 11, 96, and 99% was recorded on resistance donor P 1449-2, and on elite seed parents 843B and PT 732B, respectively. Disease incidence in the segregating progenies ranged from 10 to 100%, with an operational heritability >0.90. Segregation patterns permitted identification of BC_3F_1 progenies apparently carrying one or two partially effective resistance genes from donor P 1449-2. A small subset of the BC_3F_1 progenies (18 progenies distributed across 8 of the 16 families screened) was selected for re-screening against Sg 298 in early 2006. Disease-free seedlings from one or more of the progenies in each of these selected families will then be transplanted in the field and advanced a generation by selfing and backcrossing to produce families of BC_3F_2/BC_4F_1 progeny pairs, each expected to segregate for one or more partially effective resistance genes from P 1449-2 in the background of 843B. This map-directed conventional backcrossing program is expected to produce near-isogenic lines carrying single additional downy mildew resistance genes from P 1449-2 in the genetic background of 843B.

BC_3F_2/BC_4F_1s (PT 732B × P 1449-2) × PT 732B: Of 144 BC_3F_2/BC_4F_1 progenies from [(PT 732B × P 1449-2) × PT 732B] evaluated against New Delhi pathotype, 28 progenies (19%) were resistant, while 42 and 100% downy mildew incidence was recorded on the resistance donor P 1449-2 and the recurrent parent PT 732B, respectively. Disease incidence in the segregating progenies ranged from 0 to 100%, with an operational heritability >0.90. Segregation patterns permitted identification of BC_4F_1 progenies apparently carrying one, two, or three partially effective resistance genes from the donor P 1449-2. A small subset of the BC_4F_1 progenies (23 progenies distributed across all four families screened) was selected for re-screening against Sg 298 in 2006. Disease-free seedlings from one or more of the progenies in each of these selected families will then be transplanted in the field and advanced a generation by selfing and backcrossing to produce families of BC_4F_2/BC_5F_1 progeny pairs, each expected to segregate for one or more partially effective resistance genes from P 1449-2 in the background of PT 732B. This map-directed conventional backcrossing program is expected to produce near-isogenic lines carrying one or more additional downy mildew resistance genes from P 1449-2 in the genetic background of PT 732B.

Mapping population progeny F_4 self bulks (PT 732B-P2 × P 1449-2-P1): Of 131 F_4 self bulk mapping population progenies derived from cross [PT 732B-P2 × P 1449-2-P1], which were evaluated against the more virulent new Patancheru pathotype (Sg 409), none were resistant, while 80 and 100% disease incidence was recorded on parental lines P 1449-2-P1 and PT 732B-P2, respectively. The limited variation detected was less than expected based upon prior screens of the parental lines of this pearl millet mapping population. It was expected that this screen would permit QTL mapping of resistance from donor parent P 1449-2, which could be incorporated into the genetic background of four elite seed parent maintainer lines (at least two of which are now highly susceptible to this pathotype) into which stover quality QTLs from P 1449-2 are also being backcrossed. However, as no resistance genes are segregating in this mapping population with effects on Sg 409 that are large enough to warrant backcrossing, QTL mapping using this phenotyping data set has not yet been attempted.

Mapping population progeny F_4 self bulks (841B-P3 × 863B-P2): Of 160 F_4 self bulk mapping population progenies derived from cross 841B-P3 × 863B-P2, which were evaluated against the more

virulent new Patancheru pathotype (Sg 409), only 16 progenies (10%) were found resistant. Disease incidence on the parental lines was 94% on 841B-P3 and 11% on 863B-P2, while 100% disease incidence was observed on the susceptible control, 7042S.

QTL analysis of the mapping population progeny means by composite interval mapping procedures detected a major QTL on linkage group 4 (near RFLP marker locus *Xpsm*265 in the vicinity of the d_2 dwarfing gene locus) accounting for nearly 60% of the observed variation in downy mildew incidence among the mapping population progenies in this screen, with resistant parent 863B-P2 providing the favorable allele (having an additive effect that reduces downy mildew incidence by 32%). Two resistance QTLs of small effect, with favorable alleles from 841B-P3, were also detected (one each on linkage group 1 and linkage group 7).

While it may prove difficult in the short term to recombine resistance at this QTL with the semi-dwarf plant height that is preferred for hybrid seed parent lines, due to its linkage with a tall allele at the d_2 dwarfing gene locus, it will be relatively simple to backcross this gene into the genetic backgrounds of elite d_2 dwarf seed parent maintainer lines (as has already been initiated for recurrent parent ICMB 89111-P2). This can be done by making backcrosses from tall (D_2/d_2) BC_nF_1 plants (as pollen parent) onto the d_2-dwarf recurrent parent (as stigma parent), passing the resulting $BC_{n+1}F_1$ progenies through a severe greenhouse seedling screen against downy mildew isolate Sg409, and transplanting the disease-free segregates in the field (along with non-inoculated recurrent parent seedlings) for generation advance. At the end of this combined conventional greenhouse screening and morphological marker-assisted backcrossing program, it will be necessary to use a large $BC_{n+m}F_2$ population to find the expected rare individuals that have both d_2 dwarf plant height and are heterozygous for the resistance allele at the linked downy mildew resistance gene. A further generation of head-to-row advance, with conventional screening, will then allow identification of $BC_{n+m}F_3$ progenies that are homozygous for both d_2 dwarf plant height and the downy mildew resistance gene. When such plants are used as resistance donors, the downy mildew resistance allele will be tightly linked with the recessive dwarf plant height gene, and molecular marker-assisted selection combined with conventional screening will prove to be best alternative for introgressing this 863B resistance allele into other d_2 dwarf genetic backgrounds that are susceptible to Sg 409.

Mapping population progeny F_4 self bulks (ICMB 89111B-P6 × ICMB 90111B-P6): Of 206 F_4 self bulk mapping population progenies derived from cross ICMB 89111B-P6 × ICMB 90111B-P6, which were evaluated against the virulent Patancheru pathotype (Sg 409), only 17 progenies (8%) were found resistant, while 100% disease incidence was recorded on susceptible parent ICMB 89111B-P6 and 11% on resistant parent ICMB 90111B-P6. QTL analysis of this data set is in progress.

***Screening for resistance to downy mildew under field conditions*:** Screening downy mildew resistance of all breeding materials was conducted in a completely randomized block design with 3 replications, and plots of 2 rows × 4 m, using 7042S as the standard susceptible control. All screening trials were quite successful with 7042S recording 95 to 99% disease incidence. Lines scoring ≤10% incidence were considered resistant. A total of 99 inbred lines, hybrids, and/or open-pollinated varieties from 4 trials were screened, of which 34 were found resistant (Table 2.1). The details of each trial are provided below, comparing results from greenhouse screening of the same materials against the more virulent new Patancheru pathotype (Sg 409).

***841B-like inbreds*:** Thirteen 841B-like drought tolerance QTL introgression lines based on donor 863B-P2, and their two parental lines (841B-P3 and 863B-P2) were evaluated. Of the 15 trial entries, 14 were resistant in this field screen. In comparison, greenhouse screen of the same lines against

Patancheru pathotype Sg 409, only resistant parent 863B-P2 was considered highly resistant, while 841B-P3 and its thirteen near-isogenic derivatives were all considered susceptible. The observed differences between field and greenhouse screening are somewhat troubling, but indicative of the reasons that the greenhouse screen is preferred for selection purposes while the field screen is now restricted to a confirmatory role. The combined results from field and greenhouse screening of this set of inbreds indicated that pyramiding of downy mildew resistance and drought tolerance QTLs in the background of 841B (based on molecular marker-assisted selection for the linkage group 2 drought tolerance QTL from 863B and molecular marker-assisted selection or greenhouse seedling screening for the linkage group 4 downy mildew resistance QTL from 863B) will be necessary before an applied product can come out of the drought tolerance backcrossing program in this genetic background.

***843B-like inbreds*:** Of 44 inbred lines screened, including backcross derivatives of recurrent parent 843B with resistance donors ICML 22, P 7-3, and ICMP 85410, along with their parents, only 12 were resistant in the downy mildew nursery. In comparison, greenhouse screen of this same set of materials against Patancheru pathotype Sg 409 identified only 4 inbreds as resistant. In general, most of the near-isogenic single-QTL introgression lines in this set of materials did not have sufficiently improved downy mildew resistance to warrant widespread use in applied hybrid breeding programs. Resistance gene pyramiding (based upon marker-assisted selection or greenhouse screening of potted seedlings against one or more highly virulent pathogen isolates) and/or other resistance deployment strategies are required to generate materials for applied use. However, several of the materials in this set of inbreds could be used as near-isogenic host differential lines for characterizing pathogen virulence variation.

***H 77/833-2-like inbreds*:** Of 30 entries screened in the downy mildew nursery in the H 77/833-2-like inbreds trial, only 2 were considered resistant. Results of this field screen were similar to the greenhouse screen of these same materials against Patancheru pathotype Sg 409, in which only 1 resistant line was identified.

ACIAR pearl millet stover quality project trial: This trial contained a pair of near-isogenic hybrids differing for a genomic block controlling disease reaction to downy mildew and rust, two pairs of composite populations near-isogenic for normal and mutant alleles at a brown midrib gene, a pair of composite populations near-isogenic for a portion of pearl millet linkage group 7 associated with a putative stover quality QTL, and two released dual-purpose open-pollinated varieties. Of the 10 trial entries screened in the downy mildew nursery, 6 were resistant. No clear associations were observed between disease reaction and expected differences in ruminant livestock nutritional quality of the pairs of entries in this trial.

Hash CT, Nepolean T, Senthilvel S and Thakur RP

Activity 1.2.1.6.	Mapping *Helicoverpa* resistance in chickpea
Team	Sharma HC, Gaur PM, Hoisington D
Milestone	Mapping of pod borer resistance in chickpea (2007)

Development of interspecific mapping populations: Two RIL mapping populations are being developed from the interspecific crosses of C. *arietinum* × C. *reticulatum* (ICC 3137 × IG 72953 and ICC 3137 × IG 72953) for mapping of QTLs for resistance to the pod borer, *Helicoverpa armigera*. The crosses were advanced by one generation (F_1) in the field in the crop season, and by two generations (F_2 and F_3) in the greenhouse during the off-season. There are 210 RILs in the cross ICC 3137 × IG 72953 and 260 RILs in the cross ICC 3137 × IG 729353. Crosses were also made between ICCC 37 and ICC 506EB for initiating development of an intraspecific mapping population for mapping resistance to *H. armigera*.

Gaur PM, Sharma HC and Ridsdill-Smith TJ

***Evaluation of mapping population (ICC 506 × Vijay) for resistance to Helicoverpa armigera*:** The mapping population ICC 506 × Vijay (200 lines) was evaluated for resistance to *H. armigera* under natural infestation in the field. There were three replications in a randomized complete block design. Observations were recorded on leaf damage, numbers of eggs laid, larval density, number of pods, pods damaged, and grain yield. The leaf damage rating ranged from 1.0 to 8.0 in the mapping population, 1.7 in Vijay, 1.0 in ICC 506, and 3.2 in ICCC 37 (1 = <10% leaf area damaged, and 9 = >80% leaf area damaged). The overall resistance scores were 2.7 and 2.0 in Vijay and ICC 506, respectively, 5.2 in ICCC 37, and 2.0 to 6.7 in the mapping population. Percentage pod damage ranged from 6.9 to 34.8% in the mapping population, 22.2% in Vijay, 5.8% in ICC 506, and 25.6% in ICCC 37. This population is being evaluated for one more season to use this data for identifying molecular markers associated with resistance to *H. armigera*.

Evaluation of Cicer reticulatum × C. arietinum interspecific mapping populations for resistance to Helicoverpa armigera: The F_2s of the interspecific populations involving C*icer reticulatum* × C. *arietinum* (IG 72933 × ICC 3137, and IG 72953 × ICC 3137) were planted in the field, and evaluated for resistance to *H. armigera* using the detached leaf assay. There were five replications for each plant in a randomized complete block design. Data were recorded on leaf feeding (1 = <10% leaf area damaged, and 9 = >80% leaf area damaged), larval survival, and larval weight. In the mapping population based on IG 72933 × ICC 3137 (298 progenies + 3 checks), the leaf damage rating ranged from 1.7 to 8.0 in the mapping population, 3.5 in IG 72933 and 3.3 in ICC 3137, and 2.6 in ICC 506. Larval survival ranged from 24 to 99% in the mapping population, 62% in ICC 3137, 69% in IG 72933, and 65% in ICC 506. Larval weights ranged from 1.63 to 12.93 mg in the mapping population, 7.88 mg in ICC 3137, 5.23 mg in IG 72933, and 5.477 mg in ICC 506. In the mapping population based on IG 72953 × ICC 3137 (110 lines + 3 checks), the leaf damage rating was 1.7 to 6.9 in the mapping population, 3.9 in IG 72953 and 3.4 in ICC 3137, and 3.3 in ICC 506. Larval survival ranged from 49 to 92% in the mapping population, 61% in ICC 3137, 58% in IG 72953, and 56% in ICC 506. Larval weights ranged from 4.592 to 15. 318 mg in the mapping population, 9.647 mg in ICC 3137, 4.691 mg in IG 72953, and 7.552 mg in ICC 506. DNA extracted from this population has been sent to CAMBIA for genotyping.

Sharma HC, Gaur PM and Ridsdill-Smith TJ

Activity 1.2.1.7.	Mapping *Ascochyta* blight and *Fusarium* wilt resistance in chickpea
Team	Pande S, Gaur PM, Hoisington D
Milestone	Markers identified for *Botrytis* gray mold and *Fusarium* wilt resistance and validated (2005)

Phenotyping of recombinant inbred and germplasm lines for BGM resistance: Recombinant inbred lines (RILs) of chickpea developed from a cross ICCV 2/ICCV 10 × ICC 1496 were evaluated for BGM resistance under controlled environment conditions. Phenotyping of 222 F_3 RILs and 250 F_7 RILs of ICCV 2 × ICC 1496, and 254 F_3 and 250 F_7 RILs of ICCV 10 × ICC 1496 was conducted using conditions optimum for disease development. Ten-day-old seedlings of the test material along with JG 62 as a susceptible check were inoculated by uniform foliar spray of inoculum (3×10^5 conidia ml^{-1}). Inoculated seedlings were incubated in a growth room at 15°C and 100% RH. At 20 days after inoculation (DAI), disease severity was measured on a 1 to 9 rating scale (1 = >10% healthy plants, and 9 = >75% plants killed). RIL ICCX-000049-F_2 (10P)-847 derived from ICCV 10 × ICC 1496 was found to be resistant (<3 rating) to BGM. Distribution of moderately resistant (3.1 to) lines in each set was as follows: RILs of F_3 ICCV 10 × ICC 1496 - 179 lines; F_7 ICCV 2 × ICC 1496 - 78 lines; F_7 ICCV 10 × ICC 1496 - 17 lines.

A set of 59 chickpea lines that had distinct genotypic profiles were tested for BGM resistance under controlled environment conditions as mentioned above. Among these, ICC 1496, ICCV 93513, ICCV 96852, and ICCV 98503 were moderately resistant to BGM with a mean disease score of 3.1 to 5.

Pande C, Gaur PM and Kishore GK

Identification of molecular markers for resistance to Aschochyta blight: A linkage map of chickpea with 84 markers (82 SSRs and 2 ESTs) was constructed using F_2 population of an intraspecific cross of ICCV 04516 (AB resistant) and Pb 7 (AB susceptible). The map length was 724.4 cM with an average density of 8.62 cM. Three AB resistance QTLs were mapped, QTL1 on LG 3, and QTL 2 and QTL 3 on LG 4. The QTLs identified on LG 4 were validated in another population. A set of 84 RILs derived from the cross C 104 × WR 315 was genotyped using 73 SSR markers and screened for resistance to *Fusarium* wilt (FW) race-1. Forty-five markers were assigned to nine linkage groups. The gene for FW race 1 was mapped on linkage group 2. Three SSR markers, TA37, TA 200, and TR2 were closely linked to the resistance gene. The former was located at a distance of 0.4 cM, while the latter two markers were located at a distance of 3.5 cM. Efforts were made to expand the genome map of chickpea and mapping of *Fusarium* resistance genes using ICCV 2 × JG 62 RILs. The 126 RILs of ICCV 2 × JG 62 were screened for 12 additional polymorphic markers. Now the data is available for 206 markers on these RILs.

Gaur PM and Pande S

Activity 1.2.1.8	Mapping *Fusarium* wilt resistance in pigeonpea
Team	Hoisington D, Pande S, Saxena KB
Milestone	Markers for *Fusarium* wilt (*Fusarium udum*) resistance in pigeonpea, and fodder and feed quality in groundnut and pigeonpea mapped and verified in unrelated populations

***Standardization of phenotyping for Fusarium wilt resistance in pigeonpea*:** A reliable and reproducible technique for wilt resistance screening was standardized using three pigeonpea cultivars: ICP 2376 susceptible, and ICP 8863 and ICPL 87119 – resistant to wilt. Using root dip technique, the effect of inoculum concentration of *F. udum* on wilt development in these cultivars was studied. Eight-day-old seedlings raised in sterile sand were uprooted and the roots dipped in a conidial suspension of *F. udum* (Patancheru isolate) from eight-day-old shake culture. Conidial concentrations used for this study were: 1×10^4, 2.5×10^4, 5×10^4, 1×10^5, 2×10^5, and 3×10^5 conidia ml^{-1}. Root-inoculated seedlings were transplanted in pre-irrigated 15 cm diameter plastic pots filled with sterilized sand and black soil (4: 1). Minimum and maximum temperatures in the greenhouse were 18 to 20 and 26 to 29°C, respectively. Incidence of wilt in each treatment was recorded periodically up to 30 days after inoculation. Conidial concentration had a direct effect on the incidence of wilt. Incidence of wilt was highest at the conidial concentration of 1×10^5 and above. Cultivar ICP 2376 had 100% wilt, while ICP 8863 had 0% wilt, and ICPL 87119 had 20% wilt at 1×10^5 conidia ml^{-1} 15 days after inoculation (DAI). By 30 DAI, the mortality in ICP 8863 and ICPL 87119 increased to 20% and 40%, respectively. With a decrease in inoculum concentration, percentage mortality decreased and incubation period increased in all the three cultivars. Using the standardized root dip inoculation method and optimum inoculum concentration, phenotyping of recombinant inbred lines for wilt resistance from the cross ICP 2376 × ICPL 87119 and parents was carried out to map *Fusarium* wilt resistance.

Pande S and Saxena KB

Output 1.2.2	**Exploitation of wild relatives of crops for increasing the levels and diversifying the basis of resistance to insect pests and diseases**
Activity 1.2.2.1	Mechanisms, diversity, stability, and inheritance of resistance to *Helicoverpa* in wild relatives of chickpea and pigeonpea
Team	Sharma HC, Clements SL
Milestone	Mechanisms or resistance to *Helicoverpa armigera* in wild relatives of chickpea and pigeonpea studied.

***Mechanisms of resistance to Helicoverpa armigera in wild relatives of chickpea*:** In the absence of high levels of resistance to *H. armigera* in the cultivated germplasm of chickpea, we evaluated 25 *Cicer reticulatum* accessions for resistance to this pest. There were two replications in a randomized complete block design under field conditions. Under multi-choice conditions in the field, ten accessions showed lower leaf damage and lower numbers of eggs and/or larvae of *H. armigera*. Of these, IG 69960, IG 72934 and IG 72936 showed significantly lower leaf feeding than the cultivated genotypes and/or other accessions at the vegetative and reproductive stages. Larval weight was lower or comparable to that on *C. bijugum* (IG 70019) and *C. judaicum* (IG 70032) in *C. reticulatum* accessions IG 72933, IG 72934, IG 72936, and IG 72953 at the seedling stage, and on IG 69960 and IG 72934 at the flowering stage. Less than 7 larvae survived (out of 15) on IG 70020, IG 72940, IG 72948, IG 72949, and IG 72964 compared to 12 on ICC 506. Developmental period was prolonged by 3 to 8 days on *C. reticulatum* accessions compared to that on ICCC 37. Less than five larvae pupated on the *C. reticulatum* accessions (except IG 72958 and ICC 17163) compared to 11 in ICCC 37. Accessions showing lower leaf feeding and adverse effects on the survival and development can be used in increasing the levels and diversifying the basis of resistance to *H. armigera* in chickpea.

Sharma HC, Clemens SL and Ridsdill-Smith TJ

Mechanisms of resistance to Helicoverpa armigera in wild relatives of pigeonpea:. There was considerable variation in oviposition preference of the female moths on different accessions of the same species. *Cajanus albicans* (ICPW 13) and *C. scarabaeoides* (ICPW 90, ICPW 94, ICPW 116, and ICPW 137) were non-preferred for oviposition (<100 eggs per female) compared to the cultivated pigeonpea, ICPL 87 (334 eggs), whereas some of the accessions belonging to *C. acutifolius* (ICPW 2), *C. cajanifolius* (ICPW 28 and ICPW 29), *C. sericeus* (ICPW 160), *C. lineatus* (ICPW 40), *C. scarabaeoides* (ICPW 281), *Dunbaria ferruginea* (ICPW 178), and *Flemingia bracteata* (ICPW 192) were highly preferred as a substrate for oviposition (236 to 425 eggs).

Larval weights were <20 mg when reared on diets having pod powder of *C. acutifolius*, *C. sericeus* (ICPW 160), *C. scarabaeoides* (except ICPW 137, ICPW 141, and ICPW 152), *P. scariosa*, *C. platycarpus*, and *R. aurea* compared to 53.3 mg on ICPL 87 and 44.0 mg on ICPL 332. Larvae took >25 days to complete the development when reared on the artificial diet impregnated with lyophilized pod powder of *C. acutifolius* (ICPW 2), *C. lineatus* (ICPW 41), *C. sericeus*, *C. scarabaeoides* (except those reared on ICPW 125), *P. scariosa* (ICPW 207), *R. aurea* (ICPW 210), *D. ferruginea*, and *C. platycarpus* (ICPW 68) as compared to 15.7 days on ICPL 87, 23.3 days on ICPL 332, and 12.7 days on artificial control diet.

Sharma HC

Activity 1.2.2.2	Introgression of genes conferring resistance to *Helicoverpa* from wild relatives of pigeonpea and chickpea
Team	Mallikarjuna N, Gaur PM, Sharma HC, Upadhyaya HD

Wide crosses for pod borer resistance in pigeonpea: To transfer pod borer resistance genes from C. *scarabaeoides* to cultigens, crosses between resistant wild C*ajanus* and cultigens were attempted during the 2004 rainy season. The F_{1s} of the crosses, ICPW 94-P_1 ×ICP 28 P_1, ICPW 125-P_1 × ICP 26-P_1, ICPW 130-P_1 × ICP 26-P_2, and ICP 26-P_3 × ICP 14770-P_1 along with their parents were evaluated during the 2005 rainy season. Samples for DNA extraction have been collected. Thirty-eight resistant F_5 progenies were selected during 2004 rainy season. One single plant from each progeny has been selected for bi-parental mating to improve on deficient traits for resistance and productivity.

Upadhyaya HD

***Evaluation of progenies for resistance to pod borer from the secondary and tertiary gene pool of Cicer*:** Nine lines were selected from the cross ICCV 92318 × IG 72933 with less than 25% *Helicoverpa armigera* damage from 2004 screening experiment and screened in 2005 for *H. armigera* under unprotected field conditions. One line showed pod damage of 10%, and one line with 25%. In the rest of the lines, the damage was between 25-75%. Six lines were selected from the cross ICCV 92318 × IG 72934, from 2004 screening experiment which had *H. armigera* damage of 20%, one line showed a damage rating of less 10%. In the rest of the lines the damage rating was between 25-50%. Twenty one lines from the cross ICCV 92318 × IG 72937 were selected from 2004 *H. armigera* screening experiment. Two lines did not show any pod damage (0%), 2 lines had <10% damage, and 3 lines had 20 to 25% damage.

C*icer pinnatifidum* and C. *bijugum* were crossed with chickpea cultivars KAK-2, ICCV 2, ICCV 10, ICCV 92318, and JGK 1. Embryo rescue techniques were used to save aborting embryos from all cross combinations. Crosses with C. *pinnatifidum* did not produce normal green hybrids with any of the cultivars. C. *bijugum*, which has multiple disease resistance, produced green hybrid plants with KAK-2 and ICCV 2. This is a breakthrough in chickpea wide crosses, as hybrids have not been reported earlier with C. *bijugum*. Study of trichomes was undertaken on C. *bijugum* (IG 70006), C. *judaicum* (IG 70038) and C. *pinnatifidum* (ICCW 37), the wild C*icer* used in the crossing program. Two types of trichomes were observed. Type A was non-glandular, and present on C. *bijugum* (IG 70006), C. *judaicum* (IG 70038), C. *pinnatifidum* (IG 17152) and C. *arietinum*. Type B trichome had varied number of neck cells and was present on C. *bijugum* (ILWC 69), C. *judaicum* (IG 17150), C. *pinnatifidum* (ICCW 37) and C. *arietinum*. Hence according to the present study there is no major difference between the wild species and the cultivated chickpea used in the study based on the presence of type A and B trichomes.

***Wide hybridization in pigeonpea involving compatible Cajanus gene pool*:** Over 6550 plants were obtained as progeny from 287 selections made in 2004-2005 (Figure 2.1). In 31 selections, pod damage was < 20%, and many of the plants had a damage rating of <12% (Figure 2.2). Some of the selections had tan colored pods. From the total of 6550 plants 41 plants were found to be high yielders with the number of pods ranging from 400 to 900, and the number of seeds ranging from 1040 to 2300 plant^{-1}.

Mallikarjuna N and Sharma HC

Wide hybridization in pigeonpea involving incompatible gene pool: Advanced generation diploid (F_1BC_4) and tetraploid (F_4-F_5) derivatives from the cross C. *platycarpus* × C. *cajan* were screened for *H. armigera* under unprotected field conditions. The hybrids flowered between 70-73 days compared to 80 in the cultivated parent ICPL 85010, and 55 days in C. *platycarpus*. Pod set was

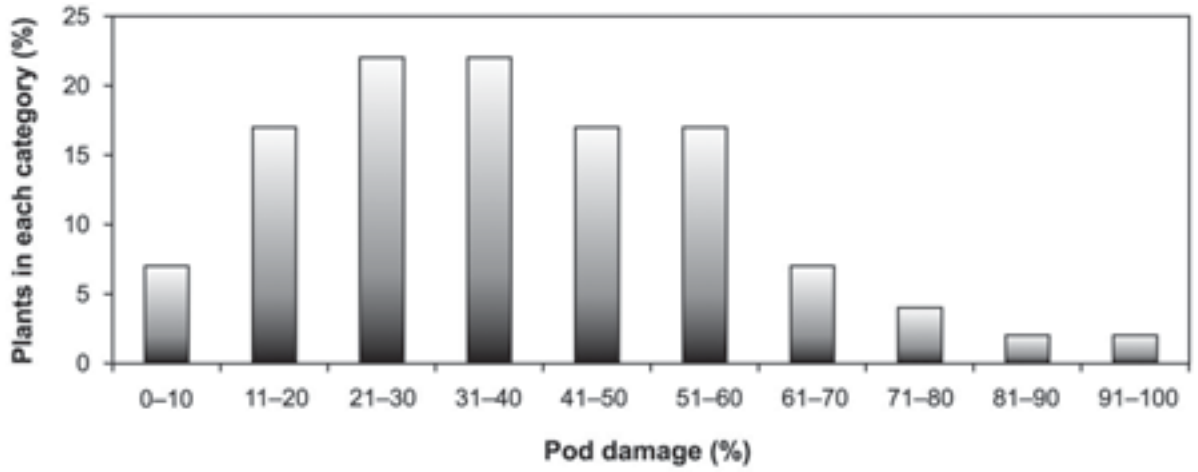


Figure 2.1. Frequencies of pigeonpea plants with different levels of Helicoverpa armigera damage in progenies derived from compatible gene pool.

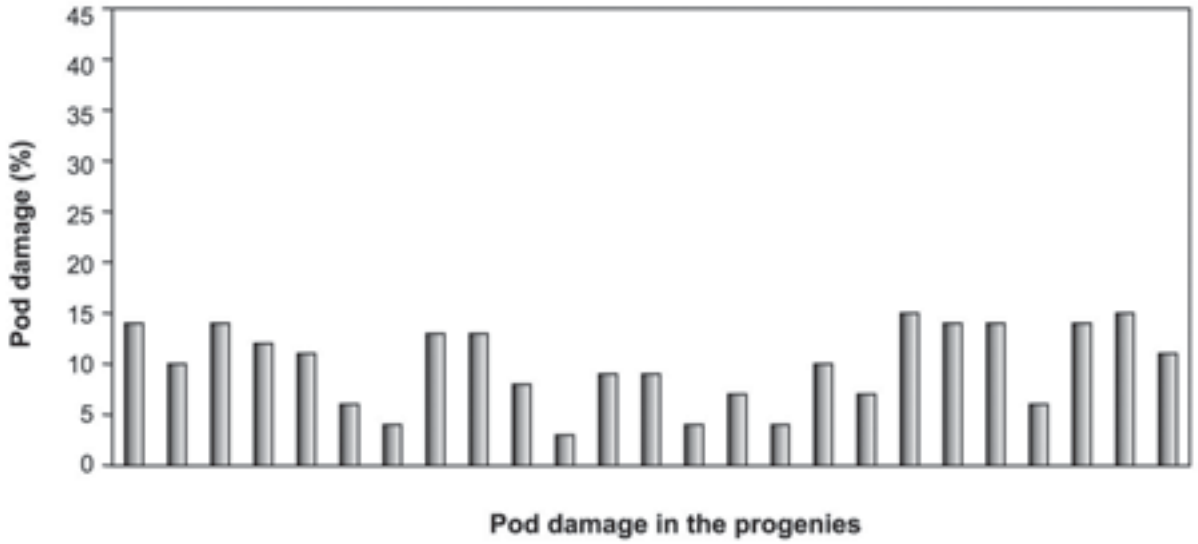


Figure 2. 2. Helicoverpa damage in plants of the progeny 8329 (Cajanus cajan cv, ICPL 2 × C. acutifolius).

earlier in the progenies when compared to cultivated parent. Damage due to *H. armigera* in the first flush, in the wild parent C. *platycarpus*, was <1%. Damage in cultivated parent ICPL 87 was 80 %. Damage in diploid derivatives was 20% and damage in tetraploid derivatives was 8%. Nearly 25% pod damage was recorded in the second and third flush in the diploid hybrids.

Mallikarjuna N and Sharma HC

***Interspecific crosses and advanced generation lines evaluated for resistance to Helicoverpa armigera*:** Selections from the interspecific crosses (derived from C*ajanus scarabaeoides*, C. *sericeus* and C. *acutifolius*) were evaluated for resistance to *H. armigera:* Forty selections derived from the crosses involving C. *scarabaeoides*, C. *lanceolatus*, C. *albicans*, C. *trinervius*, C. *sericeus* and C. *acutifolius*, along with resistant (ICPL 332, ICP 7035, and ICPL 88039) and susceptible (ICPL 87 and ICPL 87119) checks were evaluated for resistance to *H. armigera* under field conditions. There were three replications in a randomized complete block design. Data were recorded on pod damage rating,

percentage pod damage, and healthy pods. Pod damage rating ranged from 2.0 to 9.0 in the test material compared to 9.0 in ICPL 87, 4.5 in ICPL 87119, and 3.2 in ICPL 332. Selection numbers 1, 6, 7, 17, 18, 26, 33, 34, 35, and 38 had lower pod borer damage (DR <5.0, and pod damage <30%), and more number of healthy pods plant^{-1} (>50 per plant) compared to a DR of 9.0, 73% pod damage, and <15 pods plant^{-1} in ICPL 87. The ICPL 87119 had a pod damage rating of 4.5, and 44% pod damage.

Sharma HC and Upadhayaya HD

Activity 1.2.2.3.	Wide hybridization for resistance to *Botrytis* gray mold and *Ascochyta* blight in chickpea.
Team	Mallikarjuna N, Pande S, Gaur PM
Milestone	Resistance to *Ascochyta* blight and *Botrytis* gray mold introgressed in to chickpea cultivars through interspecific hybridization.

***Identification of BGM resistance in wild Cicer accessions*:** *Botrytis* gray mold (BGM) caused by *Botrytis cinerea* is a serious biotic constraint of chickpea in eastern Indo-Gangetic plains of India, Nepal, and Bangladesh. Extensive screening of germplasm and breeding material failed to detect high levels of resistance to BGM. By following standardized screening procedures, 118 wild C*icer* accessions were evaluated for résistance to BGM. Three accessions of C. *bijugum* (IG 69981, 70022, and 70023), 21 accessions of C. *judaicum* (ICCs 17148, 17149, 17151, 17193, 17194, 17204, 17205, IGs 69959, 69969, 69977, 69986, 69987, 70000, 70030, 70032, 70033, 70034, 70037, 70038, 72931, and 72932), and one accession of C. *reticulatum* (IG 72959) were found to be resistant to BGM with a mean disease score of < 2.5 on a 1 to 9 rating scale as compared to 9.0 in the susceptible check, JG 62.

Intensive screening was carried out on 155 accessions of wild C*icer* belonging to different species in 2004. From these, BGM resistant wild C*icer* accessions IG 73074 and IG 17159 *(*C. *echinospermum*) were crossed with cultivated chickpea. F_1 and F_2 plants were screened for resistance to BGM under simulated conditions. Amongst 74 hybrids derived from the cross chickpea × IG 17159, 25 had a score of 3, and 2 hybrids had a score of 2 on a scale of 1 to 9. In the cross chickpea × IG 73074, 20 hybrids were screened for BGM and 50% of the hybrids had a score of 3. These will be advanced in 2006 and screened for resistance to BGM.

Pande S, Mallikarjuna N and Kishore GK

Activity 1.2.2.4	Wide hybridization for resistance to early/late leaf spots and Aflatoxins in groundnut
Team	Mallikarjuna N, Waliyar F

A total of 105 lines of advanced generation interspepific derivatives involving *A. cardenasii*, *A. stenosperma*, *A. kempf-mercadoi* and *A. diogoi* were screened for disease reaction against late leaf spot (LLS) under field conditions during 2005 rainy season. Field trials were laid out in a broad-bed-and-furrow (BBF) system, size of each plot was 1.5 × 4 m, with inter-row spacing of 30 cm. Plant spacing of 10 cm was maintained for each line, and the experiment was un-replicated. TMV 2, a highly susceptible cultivar to LLS was used as an infector row after every five-test rows. Chemical sprays were used to control insect pests. At 50 days after sowing, plots were inoculated by spraying conidial suspension of *Phaeoisariopsis personata* urediniospores. After inoculation, perfo-irrigation was provided daily for 15 min in the evening for 30 days to create high humidity required for disease development. LLS incidence and severity was scored on a 1 to 9 rating scale at intervals of 15 days from 75 to 105 days after sowing.. Out of 113 interspecific derivatives, 19 lines from *A. hypogaea* × *A. cardenasii*, and one

line from *A. hypogaea* × *A. dura* × *A. hypogaea* showed high levels of resistance to LLS with a score of 2 (on 1 to –9) scale. From 4 wide crosses, 58 lines had a score of 3, 21 had a score of 4, Promising lines with a disease rating of 3 and less will be advanced for further testing.

In the experiment to generate interspecific amphidiploids, wild Arachis from different sections were crossed with A. hypogaea. F_1 hybrids were obtained between A. hypogaea and A. diogoi (section Arachis), BC_1 and BC_2 hybrids were obtained between A. hypogaea × A. chiquitana (section Procumbentes), and two BC_2 hybrid was obtained between A. hypogaea × A. kretschmeri (section Procumbentes).

Waliyar F and Mallikarjuna N

Output 1.2.3. **Transgenic resistance to insect pests and diseases**

Activity 1.2.3.1. Transgenic resistance to *Helicoverpa* in pigeonpea and chickpea

Team Sharma KK, Sharma HC

Milestone Transgenic plants resistant to *Helicoverpa armigera* evaluated

Putative transgenic plants carrying cry1Ac evaluated for resistance to Helicoverpa armigera under greenhouse and field conditions: Transgenic pigeonpea plants were developed by introducing the synthetic *cry1Ac* gene through *Agrobacterium tumefaciens*-mediated genetic transformation. Transgenic pigeonpea plants carrying the *cry1Ac* gene were evaluated during 2004/05 cropping seasons under contained conditions using a completely randomized block design, and there were three replications. The selected transgenic plants were bio-assayed with neonate and third-instar *H. armigera* larvae under laboratory conditions. The leaves of transgenic pigeonpea events ICPL 88039-10-3-5, ICPL 88039-15-4-2, ICPL 88039-13-1-1 (6.17 to 6.78 mg per larva) reduced the larval weight (by 40%) after 4 days of infestation as compared to that on the control plants (10.56 mg per larva). In flower bioassay, three transgenic events showed 40 to 49.2% mortality as compared to 32% on control plants. Pod bioassay of ICPL 88039-13-1-1 and ICPL 88039-15-4-2 transgenic events with third-instar *H. armigera* indicated weight gain of 156.4 to 263.2% as compared to 357.4 to 461.4% in control plants.

Evaluation of transgenic chickpea plants plants for resistance to Helicoverpa armigera: Over 20 transgenic events of chickpea with *cry1Ab* and *cry1Ac* genes were bio-assayed for resistance to *H. armigera*. Selected events and their progenies were also tested extensively under field and contained greenhouse conditions. There was approximately 35 to 40% reduction in *H. armigera* larval weight on transgenic chickpea plants as compared to control plants. In transgenic plants, larval weight ranged from 4.14 to 5.13 mg per larva as compared to 8.96 to 9.13 mg per larva on control plants. The selected plants have also been re-evaluated using detached leaf bioassay and no-choice cage technique under greenhouse conditions. The plants showing consistent biological activity will be planted under contained field conditions.

Sharma HC and Sharma KK

Activity 1.2.3.3. Transgenic groundnut resistance to fungal pathogens and viruses

Team Sharma KK, Waliyar F, Lava Kumar P

***Evaluation of transgenic groundnut for Indian peanut clump virus (IPCV) resistance*:** For the Indian peanut clump virus (IPCV), permission to carry out contained field trial of the selected 5 events of groundnut transgenic plants having coat protein or replicase genes was obtained from DBT. Contained field evaluation against IPCV was initiated at ICRISAT during the monsoon season of

2005. Five IPCV-H transgenics events IPCV cp1, IPCV cp12, IPCV cp46, IPCV rep6, and IPCV, rep21 were evaluated in a IPCV sick plot (Field# RCW17A) during 2005 rainy season at ICRISAT. Test plants along with control (JL 24) were sown in a split-plot design. Every test plant was assayed by DAS-ELISA at two weeks intervals, and plants were observed for symptoms. IPCV infection was very low in transgenic plants and also in controls (about 5%). Maximum number of infected plants were in IPCV cp46. However, two transgenic plants, IPCV cp12, and IPCV cp46, showed initial infection (tested virus positive in ELISA), but in subsequent assays were virus negative. It is likely that these two plants have potential resistance to IPCV. Due to delay in the rainy season and erratic and heavy rainfall during the plant growth season, a very low level (~10%) disease incidence was observed in the test plots, which did not allow conclusive validation of the results.

Standardization of mechanical inoculation procedure for IPCV*:* Evaluation of transgenic plants for IPCV resistance under field conditions was found to be difficult due to skewed inoculum distribution in the soil and erratic infection rate. Therefore, a reliable procedure for mechanical inoculation of IPCV was developed using innoculum prepared from IPCV-infected French bean sap extract at 1: 10 (w/v) dilution. This resulted in up to 90% infection and plants showed typical symptoms in 18 to 23 days after inoculation (Table 2.2). This procedure has proved to be very effective in evaluating IPCV resistance in transgenic plants, and is also applicable for testing resistance groundnut genotypes for resistance to this virus.

Evaluation for resistance to IPCV under greenhouse conditions: Using mechanical sap inoculation procedure, promising transgenic events IPCV cp12 (402 R1), IPCV cp1 (101-1 R2), IPCV rep6 (109-1 R3), IPCV rep21 (411-R2), and IPCV cp46 (304 R3) were evaluated for resistance (fifteen seeds per event, 3 seeds per pot). At the three-leaf stage, 12-plants were mechanically inoculated

Table 2.2 Percent transmission of IPCV (Hyderabad isolate) by mechanical sap inoculation to groundnut (JL 24).

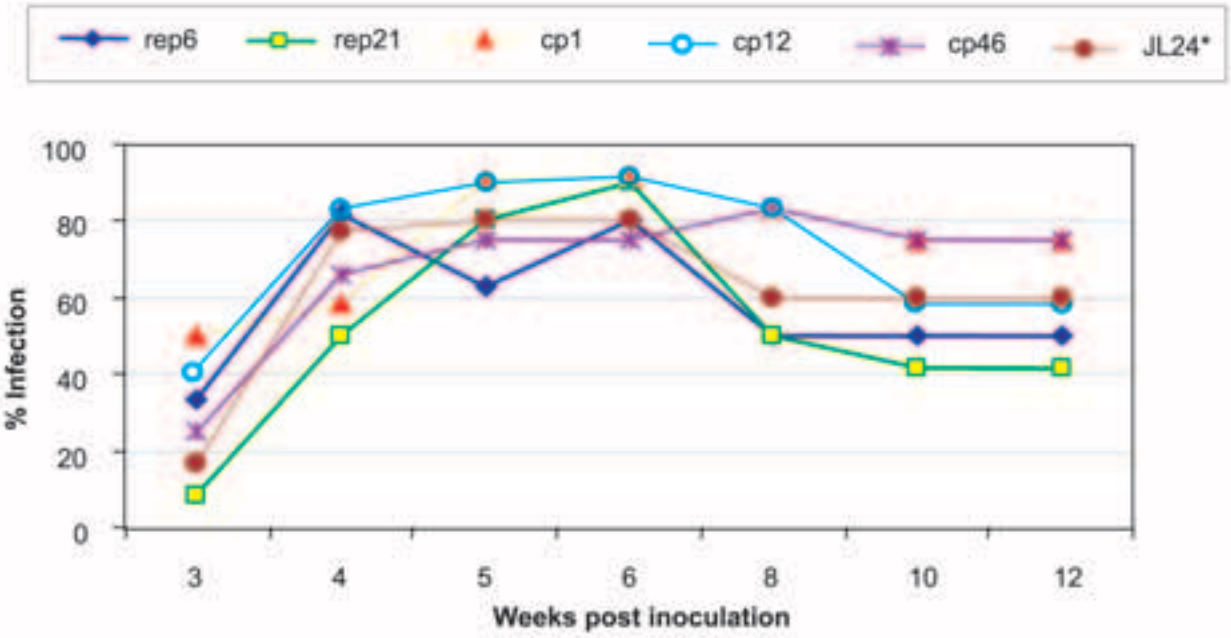
		Temperature[3]	
Incubation period[1]	Infection[2](%)	Max	Min
18	6/7 (85)	30.0	21.0
19	13/20 (65)	31.0	21.0
21	9/14 (64)	31.0	21.0
20	16/17 (94)	30.5	20.0
18	12/20 (60)	30.0	18.0
18	14/20 (70)	29.5	18.4
21	6/11 (55)	29.3	18.0
20	14/17 (82)	29.6	17.9
21	14/25 (56)	30.0	16.1
23	0/25 (0)	29.6	11.3
21	9/24 (37)	29.6	12.4
23	2/6 (33)	29.7	13.6
23	14/24 (58)	29.8	14.0
21	13/26 (50)	30.0	15.5
20	14/26 (54)	29.3	16.8
22	21/28 (75)	30.3	16.5
20.5	177/286 (62)	29.9	16.9

1 Maximum number of days at which all the virus infected plants showed symptoms;
2 Infection confirmed by DAS-ELISA; [3]Mean temperature recorded during days to infection.

with 1: 10 (w/v) extract prepared from IPCV-infected French bean. Non-transgenic JL 24 plants were used as controls. Plants were monitored for virus infection by testing newly emerging leaves at weekly intervals by ELISA. At 3 weeks post inoculation (wpi), 8 to 50% of the plants tested positive, and infection peaked at 6 wpi (75 to 92%) (Figure 2.3). At 8 wpi, virus was not detected in emerging leaves of some plants leading to reduction in percent infection Nine transgenic plants tested negative to virus. Plants, rep21 p#3 and 9; cp12 p#16; cp1 p#38, during 5 and 6 wpi tested positive to virus, but in subsequent assays, were found to be negative (Table 2.3). It is likely that these 9 transgenic plants may have good resistance to IPCV.

Lava Kumar, KK Sharma, Farid Waliyar and SN Nigam

Contained greenhouse and on-station contained field evaluation of peanut bud necrosis virus (PBNV) resistant transgenic plants: For the peanut bud necrosis virus (PBNV), the transgenic groundnut (cv. JL 24) plants of 48 independent events were produced with two vector constructs encoding nucleocapsid protein (NP) gene of PBNV through two different transformation systems. Integration of the transgene and stable genetic transformants in the T_0 and T_1 generations were assessed by PCR, RT-PCR, and Southern blot for coding regions of PBNV (NP) and *hpt*. Thirty-five independent events of T_1 generation were evaluated for resistance to PBNV under P_2 greenhouse conditions by using 100-times dilution of the virus inoculum. Of these, 24 lines showed less incidence (30%) compared to untransformed controls (100%). Transgenic plants were also evaluated in on-station contained field testing during the 2005 rainy season. Twenty-four transgenic events and 4 controls, were planted in RCBD design. Plants were sown in the month of August to take advantage of high thrip activity, and planting was done sparsely to create bare-earth effect to attract viruliferous



*All infected plants showed mosaic symptoms and stunting. IPCV incidence increased and reached peak during 6th week after inoculation, and from 8th week, virus in emerging leaves of some plants were not detected thus contributing to reduction in incidence. *JL24 is control (non-transgenic)*

Figure 2. 3 IPCV progress curve in transgenic groundnut.

Table 2.3 Promising transgenic events for resistance to IPCV.

Event	Weeks after inoculation						At harvest	Symptoms
	3	4	5	6	8	10		
IPCV rep6 (P# 71)	-	-	-	-	-	-	-	S
IPCV rep21 (P# 3)	-	-	+	+	-	-	-	NS
IPCV rep21 (P# 9)	-	-	+	-	-	-	-	SS
IPCV rep21 (P# 11)	-	-	-	-	-	-	-	SS
IPCV rep21 (P# 12)	-	-	-	-	-	-	-	NS
IPCV cp1 (P# 38)	-	-	+	+	-	-	-	MM
IPCV cp1 (P# 40)	-	-	-	-	-	-	-	S
IPCV cp12 (P# 16)	-	-	+	+	-	-	-	SS
IPCV cp46 (P# 46)	-	-	-	-	-	-	-	S

S = Stunting. NS = No symptoms. SS = Severe stunting (clump) mm = Mild mosaic no stunting. All test plants were evaluated for virus by ELISA using IPCV antibodies.

thrips (30 cm gap between plants; 50 cm gap between rows). Field trial was monitored at fortnightly intervals for symptoms and virus infection. Controls recorded 80 to 100% infection. In transgenic plants, percent infection ranged between 37.5 to 100%. Event# PBNV (B)-1 had the lowest percent infection (37.5%) at the time of harvest. (Table 2.4).

KK Sharma, Lava Kumar, Farid Waliyar and SN Nigam

***Development of transgenic plants for resistance to tobacco streak virus (TSV*):** The binary vector containing the coat protein gene of TSV (pCAMBIA 2300 TSVcp) was obtained from the Donald Danforth Plant Science Center (DDPSC) under a collaborative project supported by ABSPII. By using the *Agrobacterium*-mediated genetic transformation of cotyledonary explants, , 50 putative transgenic events were produced and transferred to the containment greenhouse. The putative transgenics were confirmed through PCR and RTPCR and Western blots, and 15 events were advanced to T_1 generation in the containment greenhouse. The recovery frequency of transgenic events was 80%.

Sai Vishnu Priya, S Arockiasam and KK Sharma

***Evaluation of transgenic groundnut containing rice chitinase gene for resistance to Aspergillus flavus by in vitro seed inoculation assay*:** Molecular characterization based on PCR of over 30 putative transgenic plants of groundnut transformed with rice chitinase gene in T_3 generation was carriedout. Preliminary bioassays with *Aspergillus flavus* (by inoculating the seeds of the selected 10 events) indictaed that 2 events had 0 to 10% incidence in terms of seed infection.

Transgenic groundnut containing rice chitinase gene was evaluated for *A. flavus* resistance by *in vitro* seed inoculation method. Seeds were inoculated with the *A. flavus* spores and incubated at 28 ºC for five days under high humid conditions. Promising T_1 events were advanced to T_2 generation. Seed from 170 plants were evaluated for *A. flavus* seed colonization. Seeds from 22 transgenic plants (event# 12, 18, 23, 24, 27, 29, 30, 31, 36, 44) that had seed infection of 0 to 10% were regenerated and advanced to T_3 (Table 2.5). Seed from 315 T_3 plants were tested for *A. flavus* seed colonization (Table 2.6). Seed infection rate in these varied from 0 to 100% (Figure 2.4). Twenty-two plants (event# 23, 27, 29, 31, 36, and 44) that had 0 to ≤10% seed colonization were selected and advanced to T_4 generation, which are under testing.

Farid Waliyar and KK Sharma

Table 2.4. On-station contained field evaluation of transgenic plants for resistance to PBNV during 2005 rainy season.

Event	Plants infected / tested	Infection (%)
PBNV (B)-10	19/23	82.6
PBNV (B)-7	17/24	70.8
PBNV (B)-6	16/24	66.7
PBNV (B)-4	16/24	66.7
PBNV (B)-3	17/23	73.9
PBNV (B)-1	9/24	37.5
Control JL 24	17/24	70.8
PBNV (B)-11	14/24	58.3
PBNV (B)-12	20/23	86.9
PBNV (B)-14	19/24	79.1
PBNV (B)-20	19/24	79.1
Control JL 24	18/24	70.8
PBNV (B)-21	20/24	83.3
PBNV (A)-A	18/24	75.0
PBNV (A)-G	21/24	87.5
PBNV (A)-F	20/24	83.3
PBNV (A)-E	19/24	79.1
PBNV (A)-D	23/23	100
Control JL 24	20/24	83.3
PBNV (A)-C	16/23	69.6
PBNV (A)-B	22/23	95.6
PBNV (A)-H	22/22	100
PBNV (A)-I	18/24	75.0
PBNV (A)-J	15/24	62.5
PBNV (A)-K	18/24	75.0
PBNV (A)-L	22/24	91.6
PBNV (A)-N	16/22	72.7
Control JL 24	19/24	79.1
SE±		2.85

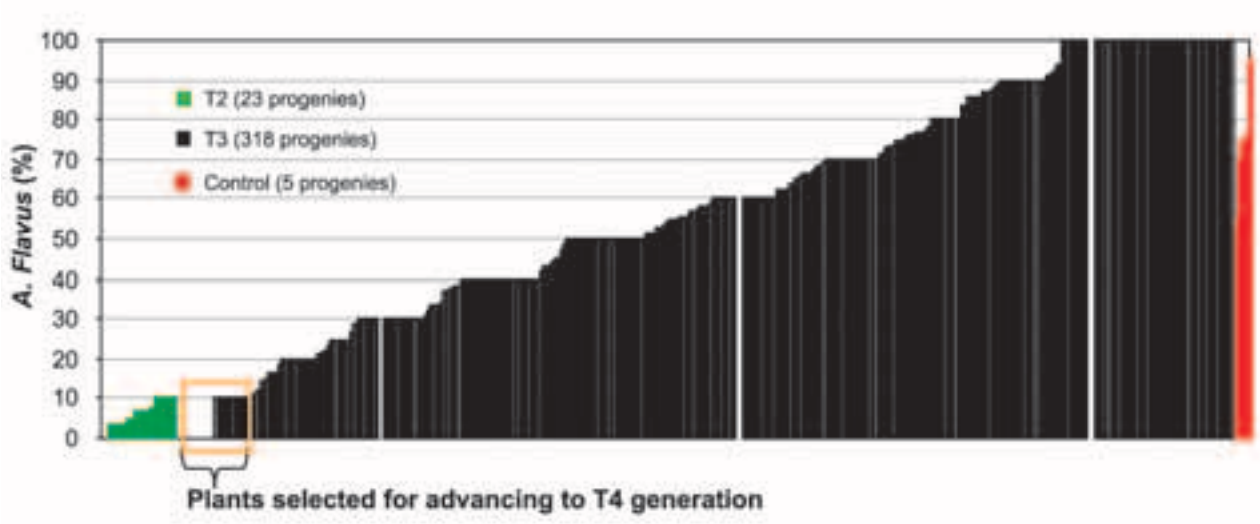


Figure 2.4. Aspergillus flavus colonization (%) in transgenic groundnut (T2 and T3).

Table 2.5. Transgenic groundnut selected for advancing from T2 to T3 generation.

Event	*A. flavus* infection(%)
12_12	0.00
18-6	3.33
29-12	3.33
36-3	3.33
44-2	3.33
44-11	3.70
27-13	4.76
23-11	5.00
23-10	6.67
29-4	6.67
29-6	6.67
30-7	6.67
44-7	6.67
29-1	7.41
29-3	8.89
23-12	10.00
24-1	10.00
27-12	10.00
30-1	10.00
30-4	10.00
31-3	10.00
36-2	10.00
Control	74.00
SE ±	0.65

Table 2.6 Transgenic groundnut selected for resistance to Aflatoxin advancing from T3 to T4 generation.

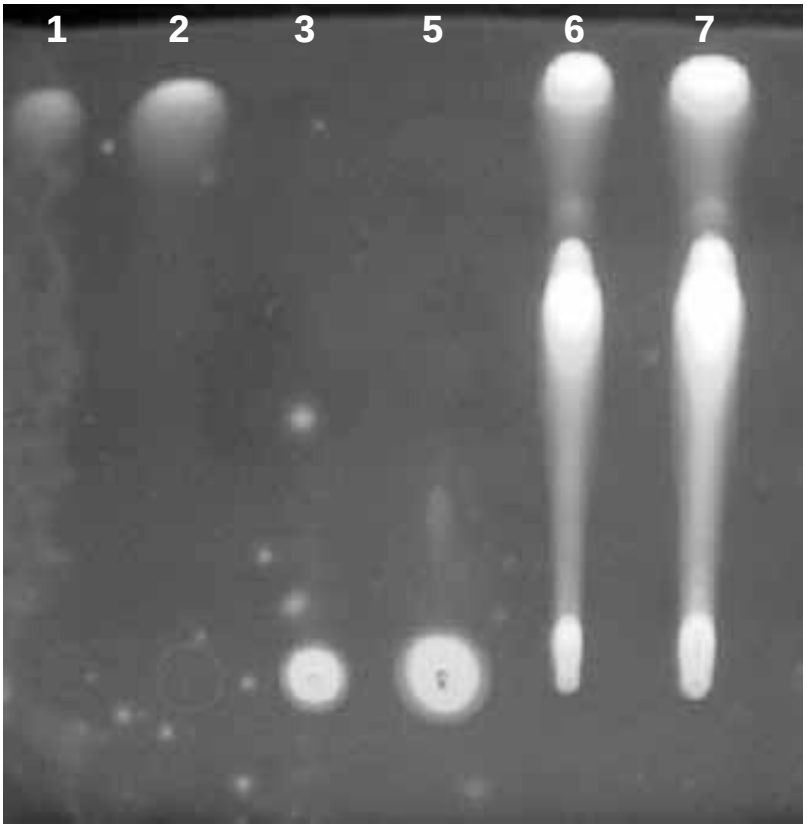
Event identity	*A. flavus* colonization (%)
23-11-1	0.00
29-3-4	0.00
29-3-5	0.00
29-6-2	0.00
29-12-1	0.00
31-3-15	0.00
31-3-17	0.00
36-2-4	0.00
36-2-14	0.00
44-7-20	0.00
44-11-2	0.00
23-12-8	10.00
27-12-4	10.00
29-6-11	10.00
31-3-16	10.00
36-2-1	10.00
36-2-2	10.00
36-2-9	10.00
36-2-15	10.00
36-2-21	10.00
44-7-14	10.00
44-7-21	10.00
Control	95.00
SE±	1.14

Output 1.2.4. **Diagnostic tools and bio-safety assessment of the products of biotechnology**

Activity 1.2.4.1 Diagnostic tools for mycotoxins and viruses

ELISA for the detection of aflatoxins in human serum: Exposure of humans and animals to Aflatoxin (AFB1) results from the consumption of contaminated food. AFB1 covalently binds to lysine moiety of serum albumin (AFB1-lys adduct). AFB1-lys adduct has been identified as a useful biomarker to determine the human exposure to aflatoxins, and to assess the exposure risk among various sections of population. For this purpose, an indirect competitive ELISA was developed for quantitative estimation of AFB1-lys adducts in human serum albumin. Protocols for preparation of standards, AFB1-lys, and AFB1-ovalbumin adducts were developed using AFB1-8,9 epoxide (Figure 2.5). These were used at various concentrations to standardize ELISA procedure using rabbit polyclonal antibodies produced at ICRISAT, and also with the antibodies obtained from the University of Columbia, USA (which was used as reference for comparative assay). A simple method for extraction of albumin from serum fraction was standardized and used for estimating AFB1-lysine concentration by ELISA. The ELISA can detect up to 1.9 pg AFB1-lys per 75 μl serum sample using reference antibody. The assay has 97.4% recovery of AFB1-lysine adduct in artificially spiked samples. However, antibodies produced at ICRISAT resulted in weak reaction and were not useful for detection of AFB1-lys adduct. ELISA test was validated using reference antibodies by analyzing Hepatitis B virus positive samples (Table 2.7). This revealed AFB1-lys levels in 4 samples (Table 2.7). This test will be validated by large scale testing of samples and new antibodies for AFB1-lys detection will be produced.

Lava Kumar and Farid Waliyar



AFB1-ovalbumin adduct has no relative mobility, and thus fluorescence appears at the spot (lanes 3 and 4); and multiple spots in organic phase (lanes 6 and 7) suggests partial and unreacted AFB1.
Lanes 1 and 2 are AFB1 standards.

Figure 2.5. Evaluation of AFB1-ovalbumin standards by thin layer chromatography.

Table 2.7 Concentration of AFB1-lysine pg mg-1 albumin (two replications).

HBV positive sample	AFB1-lys (pg mg^{-1} albumin)
1.116	0
1.165	0
1.2	0
1.20	0
0.976	30.18
1.089	0
1.198	0
1.205	0
1.198	0
1.11	0
1.042	8.5
1.162	0
1.181	0
0.98	19.42
1.024	5.14
1.121	0
1.134	0
1.121	0
1.131	0
1.081	0
Healthy control	0

***Polyclonal antibodies to PPSMV-C isolate*:** Polyclonal antibodies were produced against the C isolate. Antibody titer in DAS-ELISA is 1: 2000 for detecting the virus in leaf extracts (1: 10 w/v). These antibodies strongly reacted with a 35 kDa protein of C isolate (Figure 2.6), but not with the P isolate. Using these antibodies, it is now possible to distinguish the C and P isolates. The antibodies will be validated by testing a large number of samples.

Lava Kumar and Farid Waliyar

Activity 1.2.4.3: Characterization of pigeonpea sterility mosaic virus (PPSMV) isolates and development of diagnostic tools

Using differential pigeonpea genotypes, PPSMV isolates from southern [Bangalore (B), Coimbatore (C)], central [Dharwad (D), Gulbarga (G), and Patancheru (P)] and northern [Varanasi (V)] India were studied. The genotypes inoculated with isolates P, D, and G showed similar phenotypic reaction, and were regarded as 'P' types (Table 2.8). The genotypes inoculated with B, C, and V isolates showed similar phenotypic reaction, but distinct from the P-types, and were regarded as 'B' type. The phenotypic reaction of C isolate was similar to that of B, but they differed in size of nucleoprotein (35 and 32 kDa, respectively). The B and P isolates have similar sized nucleoprotein, but have different phenotypic reaction on differential hosts. The study indicated occurrence of several PPSMV isolates with varying degrees of properties. Based on severity, various PPSMV isolates occurring in the Indian subcontinent can broadly be grouped as B and P types. The PPSMV isolates within each group have distinct physico-chemical characteristics. The B type isolates can overcome host-plant resistance selected against P types. The B types occur in northern and southern regions, and P types occur in the central regions of India.

Lava Kumar and Farid Waliyar

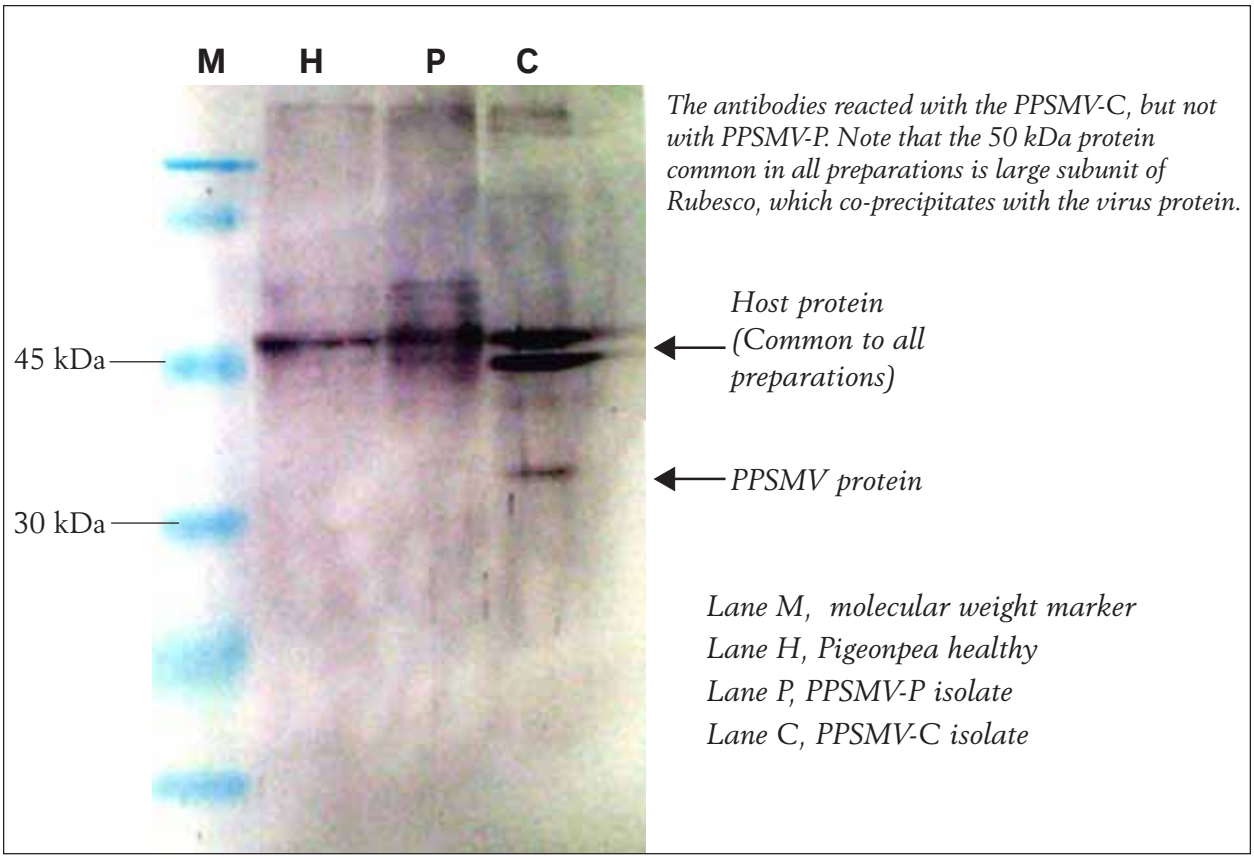


Figure 2.6. Western immuno blot assay using PPSMV-C polyclonal antibodies.

Studies on Helicoverpa armigera Nuclearpolyhedro virus (HaNPV) isolates in India: Six isolates of HaNPV obtained from different geographic locations from India were characterized [GAU-HaNPV, UASD-HaNPV, AK-HaNPV, TNAU-HaNPV, PAU-HaNPV,and ICRISAT-HaNPV]. These preparations were analyzed in 12% SDS-PAGE. Three major proteins of c 50, 32, and 30 kDa were detected in the page gels (Figure 2.7). The 50 and 32 kDa proteins were not detected in the GAU-HaNPV (Figure 2.7). The motilities of the 32 and 30 kDa protein slightly differed among various isolates. This could be due to variation in amino acid sequence or due to glycosilation. Viral DNA isolated from partially purified preparations was unsuitable for RFLP analysis. Oligonucleotide

Table 2.8. Response of pigeonpea genotypes to infection with P and Type B isolates of pigeonpea sterility mosaic virus occurring in India.

	Type P isolates			Type B isolates		
Genotype	Patancheru (P)	Gulbarga (G)	Dharwad (D)	Bangalore (B)	Coimbatore (C)	Varanasi (V)
ICP 2376	RS	RS	RS	**SM**	**SM**	**SM**
ICP 7035	NS	NS	NS	NS	NS	NS
ICP 8862	NS	NS	NS	**MM**	**MM**	**MM**
ICP 8863	SM	SM	SM	SM	SM	SM

SM = Severe mosaic. MM = Mild mosaic. RS = Chlorotic ring spots.

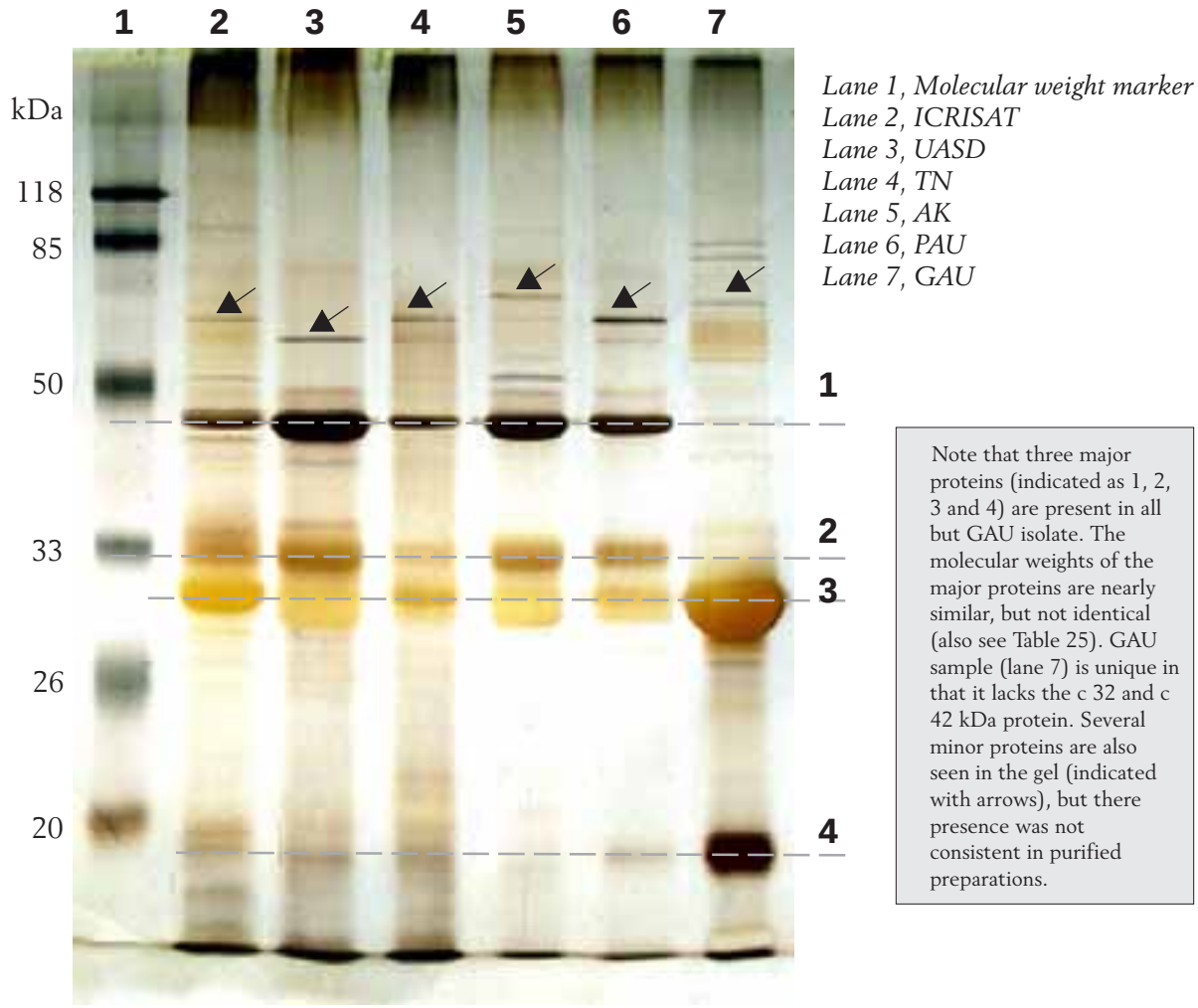


Figure 2.7. Protein profiles of different isolates of HaNPV.

primers will be designed to amplify, Bro-B gene and Hr-5 repeat region, which are known to be variable among various isolates, to study the diversity at the genome level.

Lava Kumar, GV Ranga Rao and Farid Waliyar

Activity 1.2.4.4. Non-target effects of transgenic crops on beneficial natural enemies of crop pests

Team Sharma HC, Sharma KK

Effect of Bt toxins and transgenic plants on the survival and development of the parasitoid, Campoletis chlorideae, and the coccinellid predator, Cheilomenes sexmaculatus, and other non-target insects. Four *Bt*-transgenic and the non-transgenic cotton genotypes were planted in a replicated trial under field conditions to have a better understanding of risk assessment approach for non-target arthropods. There was <10% parasitization of *H. armigera* eggs by *Trichogramma* spp. in both transgenic and nontransgenic cotton genotypes. The larval and pupal periods were longer, and larval weight and adult emergence of C. *chloridae* was lower on transgenic cotton genotypes (RCH 2

and Mech 12) as compared to their nontransgenic counterparts. A total of 40 insect specimens (25 from transgenic and 15 from non-transgenic cotton) were colleted, and were tested for the presence of *Bt*-toxin using qualitative ELISA. Among the 25 insect species colleted from transgenic plots, 7 showed high levels of *Bt*-toxin, 9 showed low levels, and the remaining 9 had no *Bt*-toxin. The transgenic cotton genotypes under sprayed and unsprayed conditions had more number of maturing bolls, good opened bolls, and lower damage in the green fruiting bodies by *H. armigera* than their nontransgenic counterparts at maturity.

Cry1Ac (LC_{50}) intoxicated *H. armigera* larvae increased the larval period of C. *chlorideae* by two days, and reduced the cocoon formation, adult emergence, and adult weight. There was no effect of *Bt* toxins on the fecundity of C. *chlorideae*. The adverse effects of *Bt* toxins on C. *chlorideae* were through early mortality of *H. armigera* larvae, and not through direct effects of the *Bt* toxins, since no traces of the toxins were found in the C. *chlorideae* cocoons and adults with the ELISA test. Studies on the host preference of *H. armigera* and it's larval parasitoid, C. *chlorideae* among six host crops of *H. armigera* (cotton, groundnut, chickpea, pigeonpea, sorghum, and pearl millet) indicated greater recovery of *H. armigera* larvae on pigeonpea and chickpea. But, chickpea was the least preferred host of C. *chlorideae* for the parasitization of *H. armigera* larvae. There was significant influence of host insects (*H. armigera*, *H. assulta*, *Spodoptera litura*, *S. exigua*, *Achoea janata*, and *Mythimna separata*) on the developmental biology of C. *chlorideae*. Two other insect species tested as xxxx (C*orcyra cephalonica* and *Sesamia inferens*) were not parasitized by the parasitoid, C. *chlorideae*. The larval period was significantly longer on *A. janata*, *S. exigua*, and *M. separata* (10.5 to 11.7 days) as compared to those reared on *H. armigera*, *H. assulta*, and *S. litura* (8.1 to 8.5 days). C*ampoletis chlorideae* had longer pupal period on *A. janata* than that on *H. armigera*. The cocoon formation and adult emergence of the parasitoid were also highest on *H. armigera* (81.6 and 70.1%) than that on other hosts. The culture of the coccinellid predator, C*heilomenes sexmaculatus*, and its aphid host, *Aphis craccivora*, has been established in the greenhouse to initiate studies on non-target effects of *Bt* toxins on generalist predators in the ecosystem. An experiment was also conducted on the mating behavior of C. *chlorideae*. There were no significant differences in parasitism efficiency of the mated or unmated females. However, the unmated females produced only male offspring.

Sharma HC and Dhillon MK

Compatibility of Bt transgenic plants with the entomopathogenic fungi: In an attempt to study genetic diversity and identify virulent strains of entomopathogenic fungi for pest management, we evaluated several fungal isolates for *H. armigera* management in collaboration with Andhra University, Vishakhapatnam, Andhra Pradesh, India. The BB 2 isolate of *Beauveria bassiana* (1×10^7 conidia per ml) in combination with neem oil (0.3%) resulted in 87.7% mortality of *H. armigera* neonate larvae (74.4% larvae with mycosis) compared to 42.2% mortality with with neem oil alone, and 70% with *B. bassiana* alone. In another experiment, BB 2 and ITCC 4688 strains of *B. bassiana* and PADP 11 and PADP strains of *Nomuraea rileyi* were evaluated for their bio-efficacy in combination with an emulsifier, rapeseed oil, linseed oil, trehalose, skim milk powder, and carboxy methyl cellulose. The *B. bassiana* strain ITCC 4688 in combination with emulsified rapeseed oil resulted in 93.66% mortality of second-instar larvae of *H. armigera* compared to 85.33% mortality with the fungus conidia alone. The *N. rileyi* strain PADP 11 in combination with emulsifiable linseed oils resulted in 89.9% mortality of second-instar larvae of *H. armigera* compared to 86.66% mortality with the fungus alone. However, maximum mycosis (95.45%) was observed with fungus conidia in

carboxy methyl cellulose. In case of tobacco caterpillar, *S. litura*, maximum mortality (91.5%) of the second-instar larvae was observed with *B. bassiana* PADP 11 conidia with emulsified linseed oil compared to 81.5% mortality with the fungal conidia alone, while maximum mycosis (90.17%) was observed with fungal conidia in carboxy methyl cellulose and emulsifier.

Uma Devi and Sharma HC

Global Project 3

Genetic Diversity, Genomic Resources and Bioinformatics

[Project Coordinator: HD Upadhyaya]

Output 1.3.2	**Molecular characterization and validation of mini-core germplasm collections**
Activity 1.3.2.1	Phenotypic and genotypic diversity assessment of the chickpea and groundnut mini-core collections
Team	Upadhyaya HD, Dwivedi SL, Gaur PM, Hoisington D, Varshney RK, Chandra S, Gowda CLL, Bhattacharjee R
Milestone	Phenotypic and genotypic information on chickpea and groundnut composite collection

***Chickpea Composite Collection Genotyped*:** A composite collection of 3000 accessions representing wide spectrum of chickpea genetic diversity (biologically - 80% landraces, 11% advanced lines/cultivars, and 1% wild species and geographically – 30% from South and South-East Asia, 25% from West Asia, 22% from Mediterranean, and 5% each from Africa and America's) was developed (Global Theme – Biotechnology Archival Report 2004). The composite collection has been genotyped using high throughput assay (ABI3700) and 35 ploymorphic SSRs at ICRISAT and 15 ploymorphic SSRs at ICARDA. Data from ICARDA is awaited. The genotypic data will be used to develop a reference collection consisting of 300 accessions (10% of the composite collection) for association mapping, functional genomics, gene tagging and genetic enhancement in chickpea.

A C (language) program was developed for allele binning based on the Idury and Cardon (1977) algorithm to provide a statistical measure of the fit of the determined "raw" alleles to an expected size based on SSR repeat unit. Using this test, we found that marker TAA58, TA21, and TR28 deviated significantly (as determined by marker quality index and allelic drift) to the expected fit, and will be dropped from the analysis. The entire dataset was formatted into a binary matrix (structure format) as well, a format necessary to operate in DARwin 5 program.

The 35 SSR loci's produced 1182 alleles, ranging between 15 and 58 alleles with an average of 33.77 alleles per SSR locus (see figure 3.1 for allelic distribution of accessions over 35 SSR loci). The polymorphic information content (PIC) for the SSR loci varied from 0.474 to 0.956, with a mean of 0.839. From the allelic distribution among accessions, several SSR loci produced unique alleles in a number of accessions. For example, SSR marker NCPGR19 in ICC 9330 (456bp) and ICC 9676 (464bp); NCPGR6 in ICC 4849 (361bp); TA200 in ICC 13912 (355bp), IG 73087 (358bp), and ICC 4853 (367bp); TA206 in ICC16915 (425bp), ICC1124 (431bp), and ICC7554 (437bp); TA21 in ICC 9402 (419bp); TA28 in ICC 12283 (438bp); TA71 in ICC 95 (139bp); TaaSH in ICC 4853 (496bp); and TR43 in ICC 10466 (417bp), ICC 4075 (432bp), ICC 228 (468bp), and ICC 4853 (474bp) produced alleles that were present only on these but absent in the remaining accessions. Further analysis is in progress to determine the population structure and identify the most diverse accessions for developing the reference collection of 300 accessions.

***Chickpea Composite Collection Phenotyped*:** Chickpea composite collection consisting 3000 accessions including core/mini core collection, was phenotyped for grain yield and related traits in an augmented design with five control cultivars during 2004-05 postrainy season. ICCs 8318, 17256,

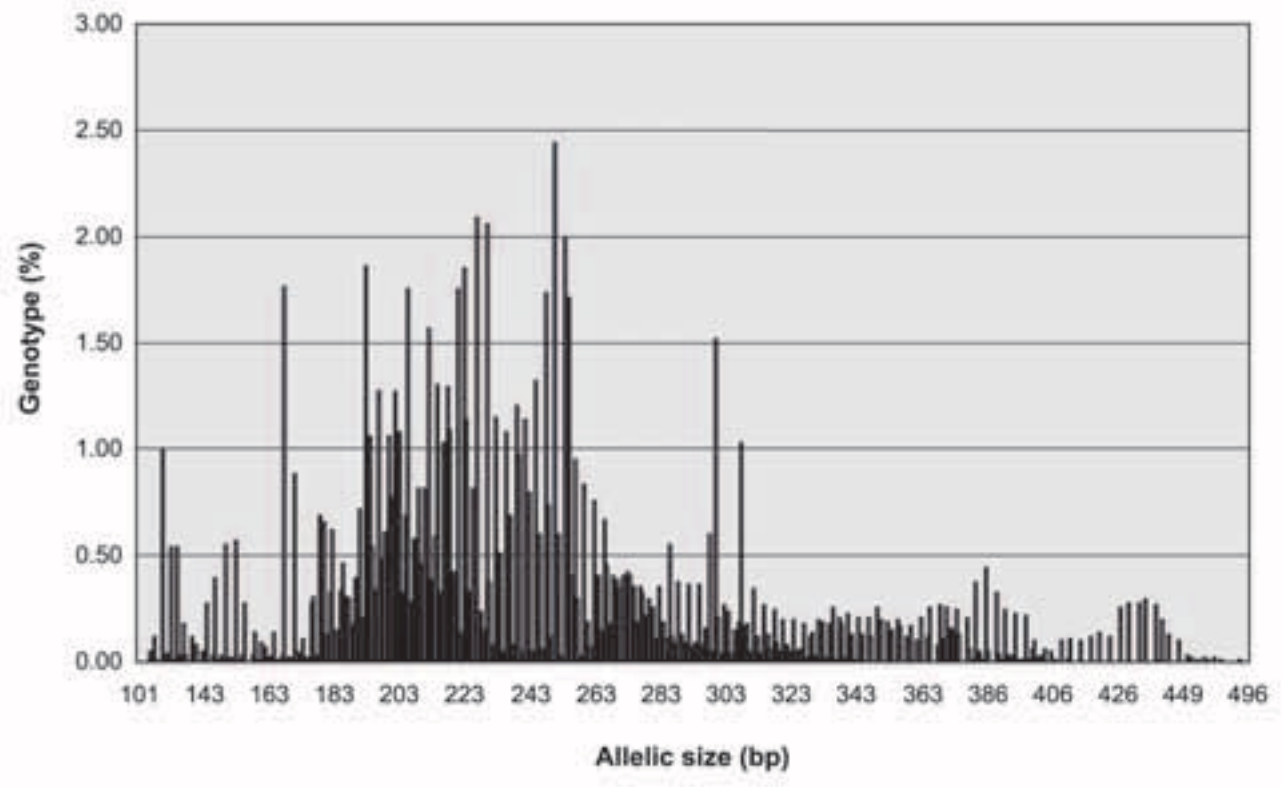


Figure 3.1. Histogram of allele frequencies for the 1182 alleles detected using 35 SSR loci in 3013 accessions.

8324, 12197, 812, and IG 70779 (2.74-3.35 t ha^{-1}) were the top high yielding accessions. ICCs 12034, 13821, 16641, 17258, 5810, and ICCVs 96329 and 96030 (33-36 days to 50% flowering, 1.18-2.02 t ha^{-1}) were the earliest flowering accessions. ICCs 12034, 7346, and 14205 (45.0-45.7 g 100^{-1}seed weight and 1.18-2.02 t ha^{-1} grain yield) among kabuli types and 14648, 4871, and 7672 (29.2-35.4 g 100^{-1}seed weight and 1.25-2.26 t ha^{-1} grain yield) among the desi types were identified as the large seeded accessions.

Upadhyaya HD, Dwivedi SL , Gaur PM, Hoisington D, Varshney RK, Chandra S and Gowda CLL

***Groundnut Composite Collection Developed*:** Groundnut composite collection consisting of 850 accessions from ICRISAT and 150 from EMBRAPA representing entire collection ecologically, taxonomically, and phenotypically was developed. The composite collections includes accessions resistance to - *A. flavus* seed colonization (16), bacterial wilt (5), bud necrosis (7), peanut mottle virus (2), rosette (12), early leaf spot (7), late leaf spot (14), rust (15), stem and pod rot (9), multiple resistant (37), aphid (1), jassid (8), leaf miner (5), thrips (4), termite (5), and accessions of - mini core collection (184), mini core comparators (184), mini core for Asia region (50), Asia core (60), elite/released cultivars (36), and drought tolerant (18), fresh seed dormancy (6), high and low biological nitrogen fixation (9) high shelling percentage (10), high oil content (5), high protein content (5), interspecific derivates (5), large seeded (10), morphological variants (26), early maturity (25), accessions genotyped earlier (18), and 52 accessions of 14 wild *Arachis* species from ICRISAT, and 62 wild accessions and 88 cultivated from Empresa Brasileira de Pesquisa Agropecuária (Brazilian Agricultural Research Corporation EMBRAPA).

***Groundnut Composite Collection Genotyped*:** 850 accessions of the composite collection were planted and leaf material was used to extract DNA for further genotyping. Twenty SSR primer pairs

were pre-screened on mini-core accessions to identify 10 polymorphic primers. At EMBRAPA, ten other polymorphic primers have been identified and these selected 20 SSR primers are being used to fingerprint the entire composite collection through capillary electrophoresis using ABI 3700. GeneScan and Genotyper softwares are used to analyze the electrophoresis data to get information on alleles. This dataset will be used for further analysis to assess genetic diversity and then a reference collection (300 accessions) of most diverse lines will be established that can be used for further genotyping and phenotying studies and in the improvement programs.

***Groundnut Composite Collection Phenotyped*:** Groundnut composite collection consisting of 850 accessions from ICRISAT was phenotyped for pod yield and related traits in an augmented design with four control cultivars, during 2005 rainy season. ICGs 6201, 6407, 6703, 10566, and 15042 were early flowering (19 days) in comparison to control cultivar ICGS 44 (22 days). ICGs 3027, 8352, 8285, 13920, and 13916 produced high pod yield (4.40 – 4.82 t ha^{-1}) in comparison to control cultivar ICGS 76 (4.38 t ha^{-1}) and ICGS 44 (3.88 t ha^{-1}). ICGs 5195 and 11427 were identified with high shelling turn over (82-83%) in comparison to ICGS 44 and ICGS 76 with 80%. ICGs 5016, 4304, 8305, 8352, and 12059 were large-seeded (87 – 89 g 100 seed weight) accessions

Upadhyaya HD, Bhattacharjee R, Hoisington D and Varshney RK

Activity 1.3.2.2	Phenotypic and genotypic diversity assessment of the sorghum, pearl millet, and finger millet core collections
Team	Upadhyaya HD, Senthilvel S, Chandra S, Hash CT, Gowda CLL, Hoisington D, Varshney RK, Jayashree B, Rizvi SMH
Milestone	Phenotypic and genotypic information on sorghum and finger millet composite collection

***Sorghum Composite Collection Genotyped*:** Leaf samples of 2300 sorghum accessions were collected for DNA extraction. Fingerprinting with 50 SSR markers is in progress.

Finger Millet Composite Collection Developed: Finger millet composite collection consisting of 1000 accessions representing entire collection ecologically, taxonomically, and phenotypically was developed with the support of Generation Challenge Program. The composite collection includes accessions – core collection (622), plant compactness and aspect (114), core collection of AICSMIP (50), grain size (5), disease resistant and good plant aspect (76), morphological variance (9), early flowering (9), nutritional quality (12), grain yield (19), grain yield and size (9), grain size (13), fodder yield (8), finger blast (2), neck blast (3), neck and finger blast (4), finger length (17), finger number 9), harvest index (5), number of productive tillers (7), and short plant height (7). During 2006 this composite collection will be genotyped at 20 SSR loci and information will be used to determine population structure and identify a reference collection of 300 accessions.

***Finger Millet Composite Collection Phenotyped*:** Composite collection of finger millet consisting 1000 accessions including core collection was phenotyped in an augmented design with four repeated control cultivars for, traits related to grain yield during 2005 rainy season. Data processing is in progress. The preliminary investigation reveals that 65 germplasm accessions were promising for seed yield than the control cultivars. The early flowering accessions were IEs 4442, 4702, 6013, 588, and 4759 (42-51 days).

Upadhyaya HD, Senthilvel S, Chandra S, Hash CT, Gowda CLL, Hoisington D and Varshney RK

Milestone	Phenotypic information on pearl millet, finger millet, and foxtail millet core/ mini core collection

Pearl Millet Core Collection Phenotyped: Pearl millet core collection consisting of 504 accessions was phenotyped for grain yield and related 15 characters during 2005 rainy season. The preliminary results reveals that IP 9496 flowered in less than 45 days and IPs 10423 11937, 11947 and 17435 flowered very late (>125 days). IP 15220 and IP 10401 grew to a height of less than 40 cm. IP 15304 and IP 15257 produced 6 and 8 productive tillers. IP 5416, IP 12310 and IP 5447 produced panicles more than 65 cm long and more than 25 mm thickness. Four accessions, IP 6510, IP 6530, IP 6554 and IP 11947 scored 8 for green fodder yield potential. Data processing is in progress. Same set was planted during 2005-06 postrainy season to validate the observations.

Finger Millet Core Collection Phenotyped: Finger millet core collection consisting of 622 accessions was phenotyped in an augumented design experiment with four control cultivars, for grain yield potential and quality characters during 2004 rainy season. IEs 2288, 3280, 3952, 5066, and 5179 (2.04-2.15 t ha^{-1}) were the high yielding accessions and IEs 501, 2322, 2957, 4759, and 6013 (49-52 days flowering and 1.29-1.51 t ha^{-1} seed yield) were early flowering accessions.

***Foxtail Millet Core Collection Developed and Phenotyped*:** Foxtail millet core collection consisting of 155 accessions was developed and phenotyped in a replicated Alpha designed trial, for grain yield and related traits during 2005 rainy season. Data processing is in progress. ISe 1254, 1227, 1234, 1286, and 1161 were early flowering (25-33 days) accessions. The preliminary investigation reveals that 16 germplasm accessions were promising for seed yield.

Upadhyaya HD, Gowda CLL, Hash CT and Hoisington D

Milestone — Technology for trait-specific mining of novel alleles and genes in sorghum, pearl millet, chickpea and groundnut germplasm collections developed and utilized

During 2005, in collaboration with researchers at the International Centre for Genetic Engineering and Biotechnology (ICGEB) and the Central University of Hyderabad, an attempt was made to generate gene-based markers associated with a pearl millet drought tolerance QTL, mapping in linkage group 2, and mapped from donor PRLT 2/89-33. This was done by isolating RNA samples from flag leaves of pairs of hybrids near-isogenic for the drought tolerance QTL allele(s) (i.e., 843A × H 77/833-2 and 843A × ICMR 01029), which had received either a fully-irrigated control treatment or a drought stress treatment initiated at panicle emergence in a greenhouse dry-down experiment. Using RNA samples collected from stressed plants of the two hybrids, a subtractive cDNA library was developed. Recombinant clones were collected and EST sequences corresponding to approximately 300 uni-genes obtained. Initial annotation of the EST sequences suggests some chloroplast genome contamination of the cDNA library. So far, none of the EST sequences obtained appear to contain SSRs that might exhibit polymorphism between the near-isogenic pollinator of the two hybrids. Such polymorphic EST-SSRs (and other polymorphic EST-based markers) could be used for exploratory allele mining in the vicinity of the pearl millet drought tolerance QTL on pearl millet linkage group 2.

Hash CT, Rizvi SMH, Senthilvel S and Jayashree B

Activity 1.3.2.3 — Phenotypic and genotypic diversity assessment of the pigeonpea core collection

Team — Upadhyaya HD, Bhattacharjee R, Hoisington D, Varshney RK, Saxena KB, Chandra S

Milestone — Phenotypic and genotypic information on pigeonpea composite and core collections

Pigeonpea Core Collection Phenotyped: Pigeonpea core collection was phenotyped for grain yield and related traits in an alpha designed experiment during 2004 rainy season. Selected 19 accessions in extra-early maturity group (mean days to 50% flowering, 68 days, harvesting index 19.0%, shelling turnover 58%, and mean seed yield 580 kg ha^{-1}) in comparison to control ICPL 87 (DF 73, HI 17.1%, shelling 51.7%, and seed yield 219 kg ha^{-1}). Similarly, 5 early maturing, 6 medium maturing, and 29 late maturing high yielding accessions combined with other traits of economic importance were identified.

Pigeonpea Composite Collection Developed: Pigeonpea composite collection consisting of 1000 accessions representing entire collection ecologically, taxonomically, and phenotypically was developed. The composite collection includes accessions – minicore collection (146), minicore comparator (146), from core collection (236), superior morpho-agronomic traits (301), resistant to biotic stresses (74), resistant to abiotic stresses (14), elite/released cultivars (20), and 63 accessions of 7 wild species.

Pigeonpea Composite Collection Phenotyped: Pigeonpea composite collection consisting of 1000 accessions including mini core collection was phenotyped in an augmented design with four control cultivars, for yield potential and related traits during 2005 rainy season. Data recording is in progress.

Pigeonpea Composite Collection Genotyped: The composite collection was planted in the field and twelve plants per accession were selected for DNA extraction. DNA from these selected 12 plants per accession were pooled together mainly to capture within accession variation. Thirty SSR primer pairs were initially selected to pre-screen sixteen most diverse mini-core accessions to identify 20 polymorphic primer pairs, which will then be used to fingerprint the entire composite collection. The genotyping will be carried out through capillary electrophoresis using ABI3700. Further data analysis will be done to determine the genetic diversity and also to develop a reference collection of 300 accessions.

Upadhyaya HD, Bhattacharjee R, Hoisington D, Varshney RK, Saxena KB and Chandra S

Output 1.3.3	**Molecular characterization of trait-based germplasm**
Activity 1.3.3.2	Phenotypic and genotypic diversity assessment of stem borer, shoot fly and *Striga* resistant sorghum germplasm
Team	Sharma HC, Hash CT, Folkertsma RT, Chandra S, Upadhyaya HD, Kiambi D, Hoisington D, Sante de Villiers
Milestone	Phenotypic and genotypic information on stem borer and shoot fly resistant sorghum germplasm

During 2005, the SSR-based genetic diversity analysis of a set of 91 elite sorghum germplasm accessions was revised during drafting of the PhD thesis of Mr. S.P. Mehtre from Marathwada Agricultural University. The set of lines included 12 shoot fly resistant and 15 stem borer resistant accessions, 9 accessions resistant to both shoot fly and stem borer, 17 midge resistant accessions, and 38 agronomically elite recurrent parents for which ICRISAT previously initiated a large-scale marker-assisted backcross program for the stay-green components of terminal drought tolerance. Based upon capillary electrophoresis separation of PCR products from 20 sorghum SSR primer pairs, a total of 118 alleles were detected in this set of sorghum lines, with an average of 5.1 alleles per SSR locus, and 13 out of the 20 (65%) SSR primer pairs were able to detect a high level of polymorphism. Jaccard's similarity coefficient (which ranged from 0.28 to 1.00) between each pair of accessions was used to construct a dendrogram to determine the relationships among accessions using the un-weighted

paired group method with arithmetic averages (UPGMA). The dendogram showed clustering of the accessions by geographical origin, race and specific traits such as resistance to specific insect pests. This study confirmed that the accessions studied are genetically quite diverged with sorghum lines showing midge, shoot fly and stem borer resistance clustering in different groups. In addition, a cluster of agronomically superior recurrent parents was identified that is genetically quite divergent from each of these insect resistant clusters. However, some of the accessions with resistance to midge, shoot fly and stem borer clustered separately, suggesting that these lines might contain unexploited genetic variation for insect resistance that could be exploited in breeding program. This information will be useful for identifying elite recurrent parents for marker-assisted backcrossing programs to introgress insect resistance QTLs from the currently available mapping populations. Further, newly identified pairs of agronomically elite and genetically diverse insect resistant breeding lines could be used for developing new mapping populations to detect additional insect resistance QTLs.

Sharma HC, Hash CT, Folkertsma RT, Chandra S and Upadhyaya HD

Milestone — Phenotypic and molecular characterization of sorghum germplasm held by ECA NARS completed (2008)

Phenotypic and molecular characterization of Sorghum germplasm in East and Central Africa. Phenotypic information of germplasm provides the entry point for plant breeding efforts, providing information on responses to biotic and abiotic stresses and farmer- and market-preferred characteristics. Molecular markers provide information on the global genetic structure of the species – typically by using twenty to fifty neutral markers – and they provide the basis for association studies and determine entry points into vast germplasm collections. Once validated, information on marker-trait associations can be used for marker-assisted breeding. Through this project, standardized documentation, phenotypic and molecular characterization of sorghum accessions held as breeding material, international nurseries or as conserved germplasm in national gene banks of 8 ECA NARS is being analyzed using 20-50 SSR markers. These are part of the GCP set of high quality microsatellite markers that is being used for the survey of a global composite set of sorghum germplasm. A regional composite set of 200 accessions per country is being analyzed with 25 markers and the additional diversity will be readily placed in relation to the components of the global sorghum germplasm structure. As part of the project, capacity in high-throughput capillary based genotyping and low-cost PAGE systems is being built in collaborating NARS through a PhD studentship and hands-on-training of 8 Visiting Scientists.

A project planning meeting has been held and consensus built on the database structure, standardized methodologies for experimental design, data collection and documentation. Training on data entry and retrieval using Microsoft Access has also been conducted. A computerized inventory of sorghum germplasm held in Uganda and Sudan has been initiated and the selection of 200 and 400 sorghum accessions made respectively. A PhD student from Sudan has been recruited and is currently being trained in basic molecular techniques while awaiting registration at the University of Free State, South Africa. Planning is underway for a second project meeting to review status and conduct a Sorghum phenotyping workshop, including hands-on-training on data entry into the previously developed Microsoft Access Database.

Kiambi D, Hoisington D and Sante de Villiers

Activity 1.3.3.3 — Phenotypic and genotypic diversity assessment of sorghum germplasm varying in flowering time and stay-green/senescence at maturity

Team	Hash CT, Folkertsma RT, Vadez V, Bidinger FR, Hoisington D, Rattunde, HFW, Sagnard F, Clerget B, Chandra S, Upadhyaya HD
Milestone	Allele-mining to develop allele-specific markers for all major flowering genes in sorghum completed (2008)

Genetic materials appropriate for this activity have been included in the composite germplasm set of sorghum that is being genotyped with approximately 50 well-distributed SSR loci in the Generation Challenge Program. However, to achieve this milestone substantial special-project funding is required.

Hash CT, Folkertsma RT, Vadez V, Bidinger FR, Hoisington D, Rattunde HFW, Sagnard F, Clerget B, Chandra S and Upadhyaya HD

Activity 1.3.3.4	Phenotypic and genotypic diversity assessment of nutritional quality in sorghum and pearl millet germplasm
Team	Hash CT, Hoisington D, Reddy BVS, Ramesh S, Rai KN, Kulkarni VN, Rattunde EW, Vadez V, Upadhyaya HD

Findings from the first two years of phenotypic diversity assessment for grain micronutrient density in sorghum in HarvestPlus, the Biofortification Challenge Program, can be summarized as follows:

- Genetic variability exists for grain densities of Fe, Zn and phytates.
- Broad-sense heritabilities are high for sorghum grain densities of Fe, Zn and phytates.
- There is limited variability for beta-carotene grain density in sorghum, and all materials with detectable levels of this micronutrient in their grain have yellow endosperm. Because of this, we recommend halting conventional phenotypic diversity assessment and marker-based genotypic diversity assessment for this trait in sorghum, allowing the limited available resources to be focused on improvement of sorghum grain densities of Fe and Zn.
- The correlation of sorghum grain densities of Fe and Zn is significant and positive, which will facilitate simultaneous improvement of the grain densities of these two micronutrients.
- There are significant negative correlations of sorghum grain densities of Fe and Zn with grain yield, probably as a result of negative correlation of sorghum grain densities of these micronutrients with grain size.
- No significant interactions of genotype × managed soil fertility (NPK) level was observed for Fe, Zn and phytate grain densities in sorghum

Similarly, findings from the first two years of phenotypic diversity assessment for grain micronutrient density in pearl millet in HarvestPlus, the Biofortification Challenge Program, can be summarized as follows:

- Although there are significant genotype × environment interaction for pearl millet grain densities of Fe and Zn, there were good correlations between two seasons [Fe (r=0.66**) & Zn (r=0.69**)] and genotype rankings for Fe and Zn density were fairly consistent across environments and laboratories.
- There is wide variability for grain Fe and Zn density in pearl millet, and high grain Fe and Zn density is available in elite backgrounds. Almost all micronutrient-dense genotypes identified are derived from '*Iniari*' germplasm.
- As in sorghum, pearl millet grain densities of Fe and Zn are positively correlated, so simultaneous improvement of the grain densities of these two micronutrients should be possible.
- A rapid, low-cost, staining protocol of Fe grain density has been optimized and can now be used for inexpensive high-throughput screening of core collections of sorghum and pearl millet for

additional Fe-dense accessions. In pearl millet the aim of such screening would be to identify 'non-*Iniari*' germplasm having high grain density of Fe.

- Thus, there are good prospects to increase grain density levels of both Fe and Zn in pearl millet.

Based on the results of the first two years of phenotypic assessment of grain micronutrient densities in these two crops, it appears there are opportunities to:

- use high-throughput method to screen core collection for additional Fe-dense accessions of sorghum and pearl millet, which are then reasonably likely to also have high grain densities of Zn;
- assess effectiveness of enhancing Fe and Zn density in released OPVs by recurrent selection;
- initiate inheritance studies of grain Fe and Zn density, which can include mapping population development in pearl millet (initially using bulk segregant analysis for grain Fe density)
- and initiate studies of nutritional availability of the Fe and Zn in grains of sorghum and pearl millet genotypes that have high grain densities of these micronutrients.

Hash CT, Hoisington D, Reddy BVS, Ramesh S, Rai KN, Kulkarni VN, Rattunde EW, Vadez V and Upadhyaya HD

Activity 1.3.3.8	Phenotypic diversity assessment of sterility mosaic disease resistance in wild and cultivated pigeonpea germplasm
Team	Lava Kumar, Upadhyaya HD, Waliyar F
Milestone	Genetically diverse sources of resistance to SMD in pigeonpea identified

Evaluation of pigeonpea mini-core for Pigeonpea sterility mosaic virus resistance*:* Pigeonpea mini-core collection, comprising 146 accessions was evaluated against two isolates of pigeonpea sterility mosaic virus (PPSMV), for B-isolate at Bangalore and P-isolate at Patancheru, India. Thirty seed of each accession was sown in plastic pots in three replications and maintained in greenhouse at Patancheru. In Bangalore sowings were done in the experimental station. Plants were inoculated with respective PPSMV isolates at 2-leaf stage, and they were monitored for symptom type and percent incidence at 2 weekly intervals and scoring was based on visual symptoms and plants were also tested for virus by ELISA using PPSMV-P polyclonal antibodies. ICP 8863 was used as susceptible control.

Of 146 accessions evaluated against P-isolate, 3 accessions (ICPs 7869, 14120 and 14155) showed no infection; 8 accessions (ICP 14368, 11910, 14229, 14569, 14147, 14545, 11833 and 14471) showed 1-10% infection; and 11 accessions (ICP 14701, 14722, 15049, 14444, 14801, 14638, 14976, 13304, 14294, 11015 and 4317) showed infection between 11-30%. Rest of the accessions showed >30% infection. Five accessions (ICPs 12123, 10654, 11015, 11059 and 3046) although showed >80% infection, the genotypes expressed chlorotic ringspot symptoms, and no sterility observed in these genotypes and they can be regarded as tolerant to SMD. This experiment is still on going and final data with complete agronomic features will be presented at later date. Against B-isolate 143 accessions were tested (no germination in case of ICPs 12105, 10559 and 12596). Only 4 of 143 accessions had <30% infection against PPSMV-B isolate (ICPs 6123, 15185, 14569 and 14976) (Table 3.1). Symptoms appeared very late in all the four genotypes.

Evaluation of pigeonpea mini-core indicates narrow base of resistance to SMD in the pigeonpea germplasm. Resistant sources to B-type of isolates are much scarce. The resistant accessions provide entry point for further evaluation of genotypes for SMD resistance. All the promising lines identified in this activity will be further validated.

Lava Kumar, Upadhyaya HD and Waliyar F

Table 3.1: Pigeonpea mini-core accessions resistant to PPSMV against P and/or B isolates.

% SMD infection	PPSMV-P Isolate	PPSMV-B Isolate
0	7869, 14120, 14155	None
1-10	14368, 11910, 14229, **14569,** 14147, 14545, 11833, 14471	None
11-20	14444, 14801, 14638	6123, 15185
21-30	14701, 14722, 15049, **14976**, 13304, 14294, 11015, 4317	**14569, 14976**

Remaining accessions of pigeonpea mini-core showed >30% infection
Common sources underlined and in bold

Output 1.3.4 **Molecular characterization of gene flow**

Activity 1.3.4.2 Sorghum and pearl millet and their wild relatives in Asia and Africa

Team Sagnard F, Rattunde HFW, Rattunde EW, Folkertsma RT, Hash CT, Reddy BVS, Hoisington D, Upadhyaya HD

Milestone Sorghum crop-to-wild gene flow in Mali and Kenya

ICRISAT leads a project on the "Environmental Risk Assessment of Genetically Engineered Sorghums in Mali and Kenya". It aims at measuring the realized amount of crop-to-wild gene flow and analyzing the farmers' practices that may limit or favour the in situ genetic introgression between cultivars and wild Sorghum populations. This is a multidisciplinary project involving population geneticists, GIS/remote sensing specialists, molecular biologists, Sorghum breeders, and social scientists from ICRISAT in Mali and Kenya, from IER and the University of Bamako in Mali, from KARI in Kenya that started in march 2005.

We found numerous morphological evidence of crop/wild introgression occurring in Mali and Kenya. The crosses made on-station between several wild and cultivated Sorghums were all successful and produced normally fertile hybrids. However, it seems that the occurrence of wild Sorghums and the presence of weedy types in farmers' field fifer between regions. In the Soudano-guinean zone of South and South-West Mali, wild and weedy types are widespread in and around farmers' fields whereas weedy sorghum are rare in the Central and Northern part of Mali where they are not mentioned as an important threat for agriculture. The amount of genetic introgression may be variable due to the phenological overlap of wild and cultivated Sorghum flowering period. Our partial results show that wild Sorghum in North Mali are more genetically differentiated from cultivated Sorghum than those from the Mandé region. They also flower earlier than the landraces cultivated in the same villages

Sagnard F, Rattunde HFW, Rattunde EW, Folkertsma RT, Hash CT, Reddy BVS, Hoisington D and Upadhyaya HD

Output 1.3.5 **Assessment of phenotypic and genotypic diversity in sorghum and pearl millet in Western and Central, and Southern and Eastern Africa**

Activity 1.3.5.1 Temporal evolution of genetic diversity of Sorghum and Pearl Millet in Niger between 1976 and 2003.

Team Sagnard F, Rattunde HFW, Rattunde EW, Hash CT, Hoisington D, Upadhyaya HD

Milestone Agromorphological and genetic marker analyses of sorghum and pearl millet collection in 79 villages across Niger

ICRISAT participated in a collaborative project on Sorghum and Pearl Millet agrobiodiversity along with French Institute for Biodiversity (IFB) including IRD, CIRAD and INRA in 2004 and 2005 to compare the genetic diversity of Sorghum and Pearl Millet collections conducted at a 27 year interval, using both molecular (32 SSR) markers and agromorphological descriptors (Figure 3.2) The results were expected to provide new insights on the impact of climatic and anthropogenic changes on the evolution of crop diversity in the Semi-Arid regions.

In Niger, substantial diversity for agromorphological traits and SSR was observed in Sorghum. The genetic diversity is highly structured among races and among regions with the durra race predominant in Eastern Niger and the guinea race mainly located in the region where the annual rainfall exceeds 600 mm per year. We did not find any evidence of genetic erosion between 1976 and 2003 at the country level despite the occurrence of several drought events and the doubling of the population in Niger during the period of study. The low genetic differentiation (Fst = 0.003) of the two sorghum collections could indicate the strong resilience of agrobiodiversity in harsh environments and support the idea that *in situ* conservation by farmers is an efficient and complementary way to conserve the diversity of crop genetic resources. The data on genetic diversity observed at a more local scale will be analyzed in 2006.

Sagnard F, Rattunde HFW, Rattunde EW, Hash CT, Hoisington D and Upadhyaya HD

Activity 1.3.5.2	Structuration of Sorghum diversity in Mali in relation to the environmental gradient and the farmers' practices.
Team	Sagnard F, Rattunde HFW, Rattunde EW, Hash CT, Hoisington D, Upadhyaya HD
Milestone	Assessment of inter- and intra-varietal structures of sorghum landrace diversity using agromorphological and microsatellite markers.

ICRISAT team in Mali participates in the project "Sorghum agrobiodiversity in Mali and Burkina Faso" funded by the French Fund for Global Environment (FFEM). This project promotes plant participatory and decentralized breeding using a large amount of local germplasm as a method to conciliate crop improvement and genetic resource conservation of Sorghum in its centre of diversity.

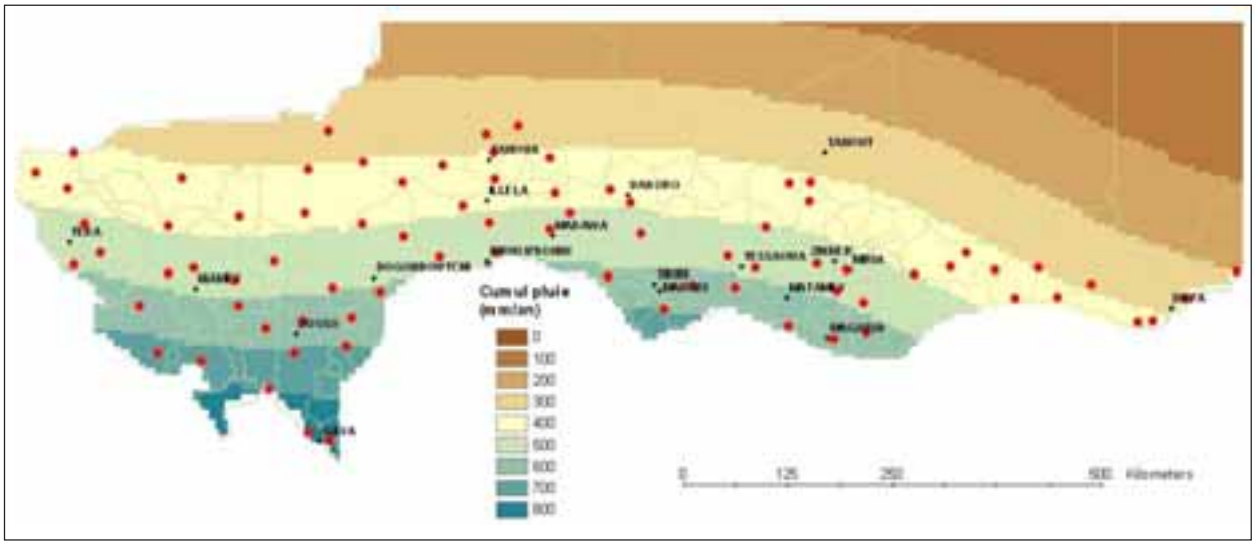


Figure 3.2 Locations of the 79 villages where Sorghum and Pearl Millet collections were undertaken in 1976 and 2003 (Bruno Gérard, ICRISAT Niamey).

The Sorghum genetic diversity is not randomly distributed in Mali along a North-South gradient. Landraces are highly structured according to botanical races and phenology. Traits such as sensitivity to photoperiod and the tillering ability are widespread in the local Sorghums. These characters should be linked to adaptation to harsh environments of the Semi-Arid tropics. The lines produced by the IER breeding program conserve an important part of the local genetic diversity. The within seed lot genetic diversity of 10 Sorghum varieties (6 guinea gambicum landraces, 3 guinea margaritiferum landraces and 1 commercial guinea gambicum variety) is more important in the guinea gambicum landraces than in the guinea margaritiferum landraces and the commercial variety (Table 3.2). An average of 1.25 to 3.36 alleles per locus were found. The heterozygote deficit is lower than values that have been previously published for the durra race sorghum except for the guinea margaritiferum landraces, which indicate a higher outcrossing rate for the guinea gambicum sorghums than generally admitted (up to 53 %).

All landraces are highly genetically differentiated (0,19 < Fst < 0,65), even among 2 landraces cultivated in the same field by the same farmer. This is much likely the consequence of repeated bottlenecks caused by sowing seeds collected on a limited number of individuals each year.

Sagnard F, Rattunde HFW, Rattunde EW, Hash CT, Hoisington D and Upadhyaya HD

Output 1.3.6 **Information management and analysis**

Team Chandra S, Jayashree B, Hoisington D

Activity 1.3.6.1 Integrated marker assisted selection system (iMAS)

Milestone Testing of standalone application integrating different software: Integrated marker assisted selection system (iMAS)

The goal of this project is to develop an integrated decision support system, called iMAS, to seamlessly facilitate marker-assisted plant breeding by integrating freely available quality software involved in the journey from phenotyping -and genotyping of genetic entities to the identification and application of trait-linked markers, and providing simple-to-understand and use online decision guidelines to use and interpret their outputs. To achieve this goal, the project has been structured into nine activities: Analyze potentially useful free software, Select software for inclusion in iMAS, Develop iMAS system, Develop and incorporate online decision guidelines, Test iMAS system, Refine

Table 3.2: Within seed lot genetic diversity of 10 Sorghum varieties from Mali assessed at 11 microsatellite markers.

Variety ID	KAG-L	DIO-A	DOUA-B	KAG-B	POM-E	SIR-B	SOUG-A	DOUA-F	DOUA-D	SIR-E
Name	CSM63E	Kalo Sabani	Nio bléni	Doubirou	Hémé piri	Sambou	Magno gnoulê nyê	Kendé blé	Kendé diéma	Kendé
Type	Guinea gambicum Commercial	Guinea gambicum Landrace	Guinea gambicum Landrace	Guinea gambicum Landrace	Guinea gambicum Landrace	Guinea gambicum Landrace	Guinea gambicum Landrace	G. marga Landrace	G. marga Landrace	G. marga Landrace
He	0,078	0,359	0,292	0,314	0,302	0,324	0,324	0,325	0,025	0,078
A	1,636	3,455	3,273	2,727	2,909	2,545	2,364	2,636	1,273	1,909
A'	1,628	3,364	3,156	2,671	2,856	2,507	2,342	2,612	1,255	1,894
Hobs	0,051	0,248	0,082	0,136	0,069	0,076	0,129	0,038	0,004	0,008
Fis	0,346	0,309	0,720	0,567	0,798	0,766	0,602	0,894	0,852	0,898
S(eq.)	0,514	0,472	0,837	0,724	0,888	0,867	0,752	0,944	0,920	0,946

He: gene diversity, A: allelic richness, A': allelic richness corrected for the sampling size, Hobs: observed heterozygosity, Fis: Wright's fixation index, S (eq.): selfing rate at the equilibrium state under a mixed mating model assumption) assed at

iMAS system, Develop iMAS user manual/tutorial, Release of and training in iMAS, and Consultation and support. The following 8 software were incorporated into iMAS, IRRISTAT, GMendel (and possibly MapDisto), PlabQTL and Win QTL-Cartographer, Tassel, PopMin, and GGT. The functionality of IRRISTAT, GMendel, PlabQTL, and Win QTL-Cartographer has been tested, problems identified, and corrective actions taken. The functionality of others is being tested. The draft text of online decision guidelines is under preparation.

Chandra S, Jayashree B and Hoisington D

Activity 1.3.6.2	Information management systems
Team	Jayashree B, Chandra S, Hoisington D
Milestone	The laboratory information management system comes into operation (2005)

Genotyping data quality algorithm developed: The workflow management system (LIMS/ Laboratory Information Management System) came into operation in 2005 to help with the capture of genotyping data from the Applied Genomics Laboratory (AGL) (can be accessed at http:// www.icrisat-intranet.org/lims/user.htm). Through the course of the year, the MS-Windows based LIMS was recoded using open source software (Java) following the interests under the Challenge Program to build platform independent applications. The database server currently runs on the Windows OS and uses the MS-SQL database for data storage, but will soon be ported to the open source PostgreSQL DB. The apache Tomcat server has been used to connect the GUI (graphic user interface) to the database. The GUI has been custom built as JSP (Java server pages) pages within the struts framework. This three-tier application is modular in its construction; incorporating four major modules: start up, sample tracking, report generation, protocols and markers. Laboratory management and data management are the two kinds of functions achieved. Being modular, the application also allows for further enhancements to be made if required, through the use, re-use or replacement of existing modules. Since the primary users of the LIMS application have been laboratory technicians and students, the goal is that the system must be easy to learn and use. The LIMS is expected to encourage best laboratory practices, through some of the data quality measures built into the application. This LIMS also incorporates automated allele binning through a Java executable of the algorithm of Idury and Cordon (1997). Testing for user acceptability of this application is currently in progress.

Genotyping information from the LIMS system flows into a larger database ICRIS (Integrated ICRISAT Crop Resources Information System). The ICRIS is envisaged to be an integrated database and currently consists of three databases for genetic resource data, genotyping data and phenotypic/ trait information. The database can be accessed through the user interface and simple reports generated from the genotyping data. Graphical views of the data in the form of histograms can also be generated. The ICRIS database incorporates the platform architecture evolving from the Challenge Program activity on generic platform development for GCP databases. It is a three-tier application and uses Hibernate for ORM (object relational mapping), to link the relational database at the back end to domain models in the middle layer. The relationships within the domain model will help the user build effective queries to retrieve information from the database through the user interface.

Jayashree B and Chandra S

Activity 1.3.6.3	High Performance Computing tool box
Team	Jayashree B, Chandra S, Elango P
Milestone	Pipeline of parallelized tools for in-silico marker discovery (2005)

The Paracel High Performance Computer through 2005: A comparative genomics and population genetics tool box consisting of parallelized versions of popular software programs is being put in place on the Paracel HPC at ICRISAT for all interested users. A number of software was configured to work on all four nodes (eight processors) of the HPC. All the tools are open-source, they include the sequence clustering tool 'MegaBlast', sequence assembly tool 'PCAP' with improvements over the original program, 'Polybayes' for SNP marker detection, 'SNP2CAPS' a tool to convert SNPmarkers to CAPS markers, and the software used in population genetics 'Structure'. Parallelization of these tools allows for analysis of larger datasets with considerable reduction in time than would be possible with single processors. Web pages to the HPC and applications installed in it have also been written using PHP (language used for server side html embedded scripting) and PBSWEb (a web based interface to the portable batch system used for job scheduling, which uses PHP) accessible at http://hpc.icrisat.cgiar.org/ and http://hpc.icrisat.cgiar.org/PBSWeb/ .

Jayashree B and Chandra S

1. List of Publications

Book

Hash, C.T. 2005. Book review - Genetic Diversity of Cultivated Tropical Plants. (Hamon, P., Seguin, M., Perrier, X. and Glaszmann, J.C. eds.), Biological Agriculture and Horticulture 23:218

Sharma, H.C. (ed.). 2005. *Heliothis/Helicoverpa* Management: Emerging Trends and Strategies for the Future Research. Oxford & IBH, New Delhi, India/Science Publishers Inc., Enfield, USA & Plymouth, UK. 469 pp.

Book chapters

Ahmad, F., Gaur P.M., and **Croser, J**. 2005. Chickpea (C*icer arietinum* L.). Pages 187-217 in Genetic Resources, Chromosome Engineering, and Crop Improvement - Grain Legumes, Vol. 1 (Singh, R.J. and Jauhar, P.P., eds). CRC Press, USA

Crouch, J.H., Gaur, P.M., Buhariwalla, H.K. Barman, P., and **Sharma, H.C.** 2005. Towards molecular breeding of Heliothis/Helicoverpa resistance in grain legumes. Pages 307-328 in Heliothis/*Helicoverpa* Management: Emerging Trends and Strategies for Future Research (Sharma, H.C., ed.). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

Crouch, J.H., Gaur, P.M., Buhariwalla, H.K., Barman, P., and **Sharma, H.C.** 2005. Towards molecular breeding of *Helicoverpa* resistance in grain legumes. Pages 313 - 334 *in Helicoverpa/Heliothis:* Management Strategies for the Future (Sharma, H.C., ed.). New Delhi, India: Oxford &IBH/Enfield, USA & Plymouth, UK: Science Publishers Inc.

Gaur, P.M., Pande, S., Sharma, H.C., Gowda, C.L.L., Sharma, K.K., Crouch, J.H., and **Vadez, V.** 2005 Genetic enhancement of stress tolerance in chickpea: Present status and future prospectus. In: Sustainable crop Production, 12 –14 Feb 2005, Indian Society of Crop Improvement, Jawahar Lal Nehru Krishi Vishvavidalaya, Jabalpur, Madhya Pradesh, India.

Gowda, C.L.L., and **Sharma H.C. 2005**. Legume pod borer/cotton bollworms, *Heliothis/Helicoverpa* - The global problem. 2005. Pages 1-9 in *Souvenir, National Symposium on* Helicoverpa *Management - A National Challenge*, 27 - 28 Feb 2005. Kanpur, Uttar Pradesh, India: Indian Society of Pulses Research and Development, Indian Institute for Pulses Research.

Kameshwara Rao, N., Mallikarjuna, N., Sharma, K.K., Reddy, L.J., Singh, P., and **Gotmare, V. 2005.** Utilization of wild relatives of crops for resistance to *Heliothis/Helicoverpa*. Pages 243 – 253 *in Helicoverpa/Heliothis:* Management Strategies for the Future (Sharma, H.C., ed.). New Delhi, India: Oxford & IBH/Enfield, USA & Plymouth, UK: Science Publishers Inc.

Kranthi, K.R, Jadhav, D.R., Kranthi, S., and **Russel, D.A.** 2005**.** Insecticidal resistance management strategies for *Helicoverpa.* Pages 405–430 *in Helicoverpa/Heliothis:* Management Strategies for the Future (Sharma, H.C., ed.). New Delhi, India: Oxford & IBH/Enfield, USA & Plymouth, UK: Science Publishers Inc.

Ntare, B.R., Waliyar F., Ramouch, Mr., Masters, E., and **Ndjeunga, J., eds. 2005.** Market Prospects for Groundnut in West Africa. CFC Technical Paper No.39. PO Box 74656, 1070 BR Amsterdam, The Netherlands: Common Fund for Commodities; and Patancheru, India: International Crops Research Institute for the Semi-Arid Tropics. 252 pp.

Ortiz, R., Ban T., Bandyopadhyay, R., Bergvinson, D., Hell, K., Jeffers, D., Menkir, A., Murakami, J., Kumar, P.L., Nigam, S.N., Upadhyaya, H.D., and **Waliyar. F**. 2005. CGIAR research-for-development program on mycotoxins. In abstracts of a conference on *Reducing Impact of Mycotoxins in Tropical Agriculture with Emphasis on Health and Trade in Africa*. Accra, Ghana. International Institute of Tropical Agriculture and Myco-Globe, pp 44-45.

Pande, S., Narayana Rao, J., Lakshmi Reddy, P. Mansur, B., and **Krishnappa, K**. 2005. Diseases of groundnut in Andhra Pradesh and their management (in Kannada). Patancheru 502324, Andhra Pradesh, India: International Crops Research Institute for the semi Arid Tropics.

Pande, S., Upadhyaya, H.D., Narayana Rao, J., Lakshmi Reddy, P. and **Parthasarathi Rao, P.** 2005. Promotion of integrated disease management of for ICGV 91114, a dual purpose, early maturing groundnut variety for rainfed areas. Information bulletin No. 68. Patancheru 502324, Andhra Pradesh, India: International Crops Research Institute for the semi Arid Tropics.

Sharma, H.C. 2005. Biotechnological approaches for management of *Helicoverpa armigera*. Pages 41-50 in *Souvenir, National Symposium on* Helicoverpa *Management – A National Challenge*, 27 - 28 Feb 2005. Kanpur, Uttar Pradesh, India: Indian Society of Pulses Research and Development, Indian Institute for Pulses Research.

Sharma, H.C., Ahmad, R., Ujagir, R., Yadav, R.P., Singh, R., and **Ridsdill-Smith, T.J**. 2005. Host plant resistance to *Helicoverpa:* The prospects. Pages 171 - 213 *in Helicoverpa/Heliothis:* Management Strategies for the Future (Sharma, H.C., ed.). New Delhi, India: Oxford &IBH/Enfield, USA & Plymouth, UK: Science Publishers Inc.

Sharma, H.C., and **Gaur, P.M.** 2005. Physico-chemical and molecular markers for host plant resistance to *Helicoverpa armigera*. In: National Symposium on *Helicoverpa* management - A National Challenge, 27 - 28 Feb 2005. Kanpur, Uttar Pradesh, India: Indian Society of Pulses Research and Development, Indian Institute for Pulses Research.

Sharma, H.C., Gaur, P.M. and **Hoisington D.A.** 2005. Physico-chemical and molecular markers for host plant resistance to Helicoverpa armigera. Pages 84-121 in Recent Advances in Helicoverpa management (Saxena, H., Rai, A.B., Ahmad, R., and Gupta, S., eds). Kanpur, India: Indian Society of Pulses Research and Development, Indian Institute of Pulses Research.

Sharma, H.C., Stevenson, P.C., and **Gowda, C.L.L**. 2005. Strategies for *Helicoverpa/Heliothis* management: Prospects and problems. Pages 479 - 485 *in Helicoverpa/Heliothis:* Management Strategies for the Future (Sharma, H.C., ed.). New Delhi, India: Oxford &IBH/Enfield, USA & Plymouth, UK: Science Publishers Inc.

Sharma, K.K., Ananda Kumar, P., and **Sharma, H.C.** 2005. Insecticidal genes and their potential in developing transgenic crops for resistance to *Helicoverpa*. Pages 261 - 280 *in Helicoverpa/Heliothis:* Management Strategies for the Future (Sharma, H.C., ed.). New Delhi, India: Oxford &IBH/Enfield, USA & Plymouth, UK: Science Publishers Inc.

Sharma, K.K., Ananda Kumar, P., Singh, N.P., and **Sharma, H.C.** 2005. Insecticidal Genes and their Potential in Developing Transgenic Crops for Resistance to Cotton Bollworm/Legume Pod Borer, *Helicoverpa armigera*. Pages 255-274 *in Helicoverpa/Heliothis:* Management Strategies for the Future (Sharma, H.C., ed.). New Delhi, India: Oxford & IBH/Enfield, USA & Plymouth, UK: Science Publishers Inc.

Stevenson, P., Pande, S., Pound, B., and **Neupane, R.K**. 2005. A strategy for wealth generation through chickpea production. Information bulletin 70. Patancheru 502324, Andhra Pradesh, India: International Crops Research Institute for the semi Arid Tropics.

Stevenson, P.C., Green, P.W.C., Simmonds, M.S.J., and **Sharma, H.C**. 2005. Physical and chemical mechanisms of plant resistance to *Helicoverpa*: Recent Research on Chickpea and Pigeonpea. Pages 215 - 228 *in Helicoverpa/Heliothis* Management Emerging trends and Strategies for the Future Research (Sharma, H.C., ed.). New Delhi, India: Oxford &IBH/Enfield, USA & Plymouth, UK: Science Publishers Inc.

Subbarao GV, Ito O, Serraj R, Crouch JH, Tobita S, Okada K, Hash CT, Ortiz R and **Berry WL.** 2005. Physiological perspectives on improving adaptation to drought – Justification for a systemic component-based approach. Pages 577–594 *in* Handbook of Photosynthesis, 2nd edition (Pessarakli M, ed.). CRC Press.

Subbarao, G.V., Ito, O., Serraj, R., Crouch, J.H., Tobita, S., Okada, K., Hash, C.T., Ortiz, R., and **Berry, W,L.** 2005. Physiological perspectives on improving adaptation to drought – Justification for a systemic component-based approach. Pages 577-594 in Handbook of Photosynthesis, 2nd edition (Pessarakli, M., ed.). CRC Press.

Urkurkar, J.S., Sharma, M.L., Pande, S., and **Gaur, P.M.** 2005. Rice based chickpea production technology. (in Hindi). Patancheru 502324, Andhra Pradesh, India: International Crops Research Institute for the semi Arid Tropics.

Conference Papers

Arora, R., Sharma, H.C., Dhillon, M.K., and **Romies, J.** 2005. Influence of Cry1Ab and Cry1Ac intoxicated *Helicoverpa armigera* larvae on the survival and development of the parasitoid, *Campoletis chlorideae*. Page 3341 *in* IVth International Food Legumes Research Conference: Food Legumes for Nutritional Security and Sustainable Agriculture, 18-22 Oct 2005. Indian Agricultural Research Institute, New Delhi, India.

Aruna, R., Nigam, S.N., and **Waliyar, F.** 2005. Current status of foliar diseases resistance breeding in groundnut at ICRISAT Center, India. Pages 63-65 *In* Summary Proceedings of International Peanut Conference 2005 "Prospects and Emerging Opportunities for Peanut Quality and Utilization Technology", January 9-15 2005, Kasetsart University, Bangkok, Thailand (Abstract).

Clement, S.L., Sharma, H.C., and **Ridsdill-Smith, T.J.** 2005. Confronting the Lepidoptera pod-borer problem on chickpea through international collaboration and research. Spotlight on 2005 Symposia: Post Session on International Affairs. Annual Meeting of the Entomological Society of America, Nov 2005, Baton Rouge, Florida, USA.

Dar, W.D., and **Upadhyaya, H.D.**. 2005. Plant genetic resources as a means for sustainable agriculture. Lead paper presented at the National Conference on Biopiracy: A Threat to National Wealth of Biological Resources, March 22-23, 2005. Osmania University, Hyderabad, India.

Desai, S., Khandar, R.R., Waliyar, F., Thakur, R.P., Dhruj, I.U., Nigam, S.N., and **Bandyopadhyay, A.** 2005. A HACCP based approach for an integrated management of aflatoxin contamination in groundnut in Gujarat, India. Pages 102 *In* Summary Proceedings of International Peanut Conference 2005 "Prospects and Emerging Opportunities for Peanut Quality and Utilization Technology", January 9-15 2005, Kasetsart University, Bangkok, Thailand (Abstract).

Girish, A.G., Sastry, D.V.S.S.R., Thakur, R.P., and **Upadhyaya, H.D.**. 2005. Seed health testing for long-term conservation and international exchange of crop germplasm. Paper presented in the Global Conference organized by the Indian Society of Mycology and Plant Pathology, 25-29 Nov 2005, Udaipur, Rajasthan, India.

Gowda, C.L.L., and **Upadhyaya, H.D.,** 2005. Changing scenario in CGIAR genebanks- Genebank's role in crop improvement- a case study of ICRISAT. Invited lead paper presented at the MDP Program of IPR, 27 July 2005, NAARM, Hyderabad India.

Gowda, C.L.L., and **Upadhyaya, H.D.,** 2005. Conservation of crop germplasm diversity at ICRISAT. In workshop to develop the regional conservation and utilization strategy for GCDT, Beijing, China, October, 15-18, 2004.

Gowda, C.L.L., and **Upadhyaya, H.D.**. 2005. Problems and prospects for enhanced use of agricultural biodiversity for crop improvement and sustainability of agricultural production. Lead paper presented at the ARS Foundation day, 25 July 2005, NAARM, Hyderabad India.

Gowda, C.L.L., and **Upadhyaya**, **H.D**. 2005. Role of science in conservation of crop germplasm diversity: the CGIAR Perspective. Invited paper presented at the Brainstorming Session on Role of Science and Society Towards PGR Management-Emerging Issues, 7-8 Jan 2005, NBPGR, New Delhi, India.

Gowda, C.L.L., and **Upadhyaya, H.D.**. 2005. Strategy for conservation and enhanced use of crop genetic resources. Paper presented in the SABRAO Congress, 22-23 August 2005, Tsukuba, Japan. p 71-80.

Gowda, C.L.L., Pundir, R.P.S., and **Upadhyaya, H.D.**. 2005. Impact of research and development in food legumes on production and productivity in the last two decades. Invited paper presented in 4th International Food Legumes Research Conference – Food Legumes for Nutritional Security and Sustainable Agriculture. 18-22 October 2005, New Delhi. In Abstracts (ed. M.C. Kharkwal) p. 4.

Halewood, M., Gaiji, S., Upadhyaya, H.D., and **Karamura, D.** 2004.Germplasm flows in and out of Kenya and Uganda through the CGIAR: a case study of pattern of exchange and use to consider in developing national policies. Paper presented at a meeting in Belliago, Italy 5-6 October 2004.

Clarke, H., Wilson, J., Croser, J., Lulsdorf, M., Mallikarjuna, N., and **Siddique, K**. 2005. Embryo rescue and plant regeneration of selfed chickpea (C*icer arietinum* L.) and its wild relatives. *In* Bennet, I.J., Bunn, E., Clarke, H., and McComb, J.A., (Eds.) ' Contributing to a Sustainable Future'. Proceedings of the Australian branch of the IAPTC&B, Perth, Western Australia, 21-24th September 2005. pp 56-64.

Jayashree, B., Leeladevi, Y., Praveen Reddy, T., Folkertsma, R., Buhariwwala, H.K., Senethilvel, S., Eshwar, K., Crouch, J.H., Chandra, S., and **Hoisington, D.** A laboratory information management system (LIMS) for genotyping data. International Conference in Plant genomics and Biotechnology: challenges and opportunities, Raipur-26th -28th Oct 2005.

Jayashree, B., Prasad, P.V.N.S., Shinde, S., Chandra, S., and **Hoisington, D.** 2005. SSR variation in coding regions among legumes (*G.max, M.truncatula, L.japonicus*) and cereals (*S.bicolor, Z.mays, O sativa*). International Conference in Plant genomics and Biotechnology: challenges and opportunities, Raipur-26th -28th Oct 2005.

Jayashree, B., Shinde, S., Prasad, P.V.N.S., Rajalakshmi, R., Chandra, S., Crouch, J.H., and Hoisington, D. 2005. A database for orthologous sequence alignments from plant environment stress cDNA libraries. International Conference in Plant genomics and Biotechnology: challenges and opportunities, Raipur-26th -28th Oct 2005.

Kashiwag, J., Krishnamurthy, L., Upadhyaya, H.D., Chandra, S., and **Gaur, P.M.**. 2005. Genetic control of drought avoidance root traits in chickpea (C*icer arietinum* L.). Invited paper presented in 4th International Food Legumes Research Conference – Food Legumes for Nutritional Security and Sustainable Agriculture. 18-22 October 2005, New Delhi. In Abstracts (ed. M.C. Kharkwal) p. 91.

Kumar, P.L. and Waliyar, F. 2005. Advances in diagnosis of eriophyid-mite transmitted viruses and virus-like agents of undefined etiology, with a particular reference to pigeonpea sterility mosaic disease. In Abstracts 2nd Global Conference on Plant Health – Global Wealth, Rajasthan College of Agriculture and Technology, Udaipur, India. *(Invited presentation)*

Kumar, P.L., Narayana, Y.D., Muniyappa, V., Rangaswamy, K.T., Jones, A.T., Waliyar, F. and Kimmins, F. 2005. Epidemiology and Management of pigeonpea sterility mosaic virus. In Abstract *IX International Plant Virus Epidemiology Symposium*. April 4-7, Lima, Peru. p52. *[By invitation]*

Kumar, P.L., Waliyar, F. and **Saxena, K.B.** 2005. Management of pigeonpea sterility mosaic disease. In Proceedings IPM Research at ICRISAT: Present Status and Future Priorities. ICRISAT, Patancheru 502 324, India. p45-51.

Mallikarjuna, N., Sharma, H.C., Jadhav, D.R., Pampapathy, G., Upadhayaya, H.D., and **Hoisington, D.** 2005. Use of wide hybridization to improve *Helicoverpa* resistance in pigeonpea [C*ajanus cajan* (L.) Millsp.]. Page 138 *in* IVth International Food Legumes Research Conference: Food Legumes for Nutritional Security and Sustainable Agriculture, 18-22 Oct 2005. Indian Agricultural Research Institute, New Delhi, India.

Mirabolfathy, M., Moradi Ghahdirjani, M., and **Waliyar, F.** 2005. Variability in Aflatoxicogenic Potential and Sclotial Production of *Aspergillus Flavus* in Pistachio of Iran. Pages 188-189 *In* IV International Symposium on Pistachio and Almond – ISHS – Tehran, Iran, May 22-25, 2005 (Abstract).

Ortiz R, Ban T, Bandyopadhyay R, Bergvinson D, Hell K, James B, Jaffers D, Mankir A, Murakami J, Kumar L, Nigam SN, Upadhyaya HD, and **Waliyar F**. 2005. CGIAR research-for-development program on mycotoxins. CABI Publications.

Pande, S., Basandrai, A.K., Kishore, G.K., Gaur, P.M., Kannan, S., Rao, J.N., Crouch, J.H., Siddique, K.H.M., and **Gowda, C.L.L.** 2005. Morphological, pathogenic and genetic variability in Indian isolates of *Ascochyta rabiei* of chickpea. *In* Proceedings of the 15th Australasian Plant Pathology Society Conference, Geelong, Australia. 26-29 September, 2005. pp. 365.

Pande, S., Neupane, R.K., Bakr, M.A., Stevenson, P., Rao, J.N., MacLeod, B., Siddique, K.H.M., Kishore, G.K., Chaudhary, R.N., Joshi, S., and **Johansen, C.** 2005. Rehabilitation of chickpea in Nepal and Bangladesh

through integrated management of Botrytis grey mould. *In* Proceedings of the 15th Australasian Plant Pathology Society Conference, Geelong, Australia. 26-29 September, 2005. pp. 252.

Ramakrishna Babu, A., Sharma, H.C., Subaratnam, G.V., and **Sharma, K.K.** 2005. Development of transgenic chickpea (C*icer arietinum* L.) with Bt *cry1Ac* gene for resistance to pod borer, *Helicoverpa armigera.* Page 58 *in* IVth International Food Legumes Research Conference: Food Legumes for Nutritional Security and Sustainable Agriculture, 18-22 Oct 2005. Indian Agricultural Research Institute, New Delhi, India.

Ramu, P., Hash, C.R., Jayashree, B., Chandra, S., Rekha, B., Prasad, P.V.N.S., Mahalakshmi, V., and Crouch, J.H. 2005. **Development and application of EST-SSR markers in sorghum for comparative genome mapping with rice". Submitted to the International Conference on "Sustainable crop production in stress environments: management and genetic options". Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.) India February 9-12, 2005.**

Reddy, P.V., Sudhakar, P., Naidu, P.H., Nigam, S.N., Waliyar, F., Nageswara Rao, R.C., and **Wright, G.C.** 2005. Selection for groundnut varieties with low aflatoxin risk under terminal moisture stress. Pages 112-113 *In* Summary Proceedings of International Peanut Conference 2005 "Prospects and Emerging Opportunities for Peanut Quality and Utilization Technology", January 9-15 2005, Kasetsart University, Bangkok, Thailand (Abstract).

Serraj, R., Hash, T.C., (*sic*), Buhariwalla, H.K., Bidinger, F.R., Folkertsma, R.T., Chandra, S., Gaur, P.M., Kashiwagi, J., Nigam, S.N., Rupakula, A., and **Crouch, J.H.** 2005. Marker-assisted breeding for crop drought tolerance at ICRISAT: Achievements and prospects. Pages 217–238 *in* Proceedings of the International Congress "In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution", 27–31 May 2003, Bologna, Italy (Tuberosa R, Phillips RL and Gale MD, eds.). Bologna, Italy: Avenue media

Serraj, R, Hash, T.C. (*sic*), Buhariwalla, H.K., Bidinger, F.R., Folkertsma, R.T., Chandra, S., Gaur, P.M., Kashiwagi, J., Nigam, S.N., Rupakula, A., and **Crouch, J.H.** 2005. Marker-assisted breeding for crop drought tolerance at ICRISAT: Achievements and prospects. Pages 217-238 *in* Proceedings of the International Congress "In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution", 27-31 May 2003, Bologna, Italy (Tuberosa, R., Phillips, R.L., and Gale, M.D., eds.). Bologna, Italy: Avenue media

Serraj, R, Hash, T.C. (*sic*), Buhariwalla, H.K., Bidinger, F.R., Folkertsma, R.T., Chandra, S., Gaur, P.M., Kashiwagi, J., Nigam, S.N., Rupakula, A., and **Crouch, J.H.** 2005. Marker-assisted breeding for crop drought tolerance at ICRISAT: Achievements and prospects. Pages 217-238 *in* Proceedings of the International Congress "In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution", 27-31 May 2003, Bologna, Italy

Sharma, H.C. 2005a. Arthropod response to genetically diverse cropping systems and its implications in pest management. Page 27, Abstracts. *National Symposium on Biodiversity and Pest Management*, 3-4 Feb 2005, Entomology Research Institute, Loyolla College, Chennai, Tamil Nadu, India.

Sharma, H.C. 2005b. Non-traget effects of transgenic crops on the natural enemies of *Helicoverpa armigera*. In: Annual Meeting of IndoSwiss Collaboration in Biotechnology, 5 Feb 2005, Kamraj University, Madurai, Tamil Nadu, India.

Sharma, H.C., **Arora, R**., and **Romeis , J**. 2005. Effect of genetically modified crops on beneficial insects. Page 35, Abstracts. *National Symposium on Biodiversity and Pest Management*, 3-4 Feb 2005, Entomology Research Institute, Loyolla College, Chennai, Tamil Nadu, India.

Sharma, H.C., Clement, S.L., Ridsdill-Smith, T.J., Ranga Rao, G.V., El Bouhssini, M., Ujagir, R., Srivastava, C.P., and **Miles, M.** 2005. Insect pest management in food legumes: Future strategies. Page 25 *in* IVth International Food Legumes Research Conference: Food Legumes for Nutritional Security and Sustainable Agriculture, 18-22 Oct 2005. Indian Agricultural Research Institute, New Delhi, India.

Sharma, H.C., Dhillon, M.K., and **Romies, J.** 2005. Biosafety of insect-resistant transgenic plants to non-target organisms. Paper Presented at the National Symposium on, "Recent Trends in Environmental Biology and Biotechnological Approaches to Conserve Biodiversity, 22 - 24 Oct 2005. Academy of Environmental Biology, Gulburga University, Gulburga, Karanataka, India.

Sharma, H.C., Dhillon, M.K., and **Romies, J.** 2005. Nontarget effects of transgenic crops in the environment. Paper Presented at the National Symposium on, "Transgenic Crops in Pest Management, 12 - 13 Sept 2005. Center for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Sreelatha, G., Sharma, H.C., Manohar Rao, D., Royer, M., and **Sharma, K.K.** 2005. Genetic transformation of pigeonpea [C*ajanus cajan* (L.) Millsp.] with *Bt cry1Ac* gene and the evaluation of transgenic plants for resistance to *Helicoverpa armigera*. Page 58 *in* IVth International Food Legumes Research Conference: Food Legumes for Nutritional Security and Sustainable Agriculture, 18-22 Oct 2005. Indian Agricultural Research Institute, New Delhi, India.

Stevenson, P.C., Pande, S., Neupane, R.K., Chowdhary, R.N., Bourai, V.A., Rao, J.N., and **Grzywacz, D. 2005.** The adoption of ICM technologies by poor farmers in Nepal. Pages 135-142 *in* Policy and strategy for increasing income and food security through improved crop management of chickpea in rice fallows in Asia. S Pande, PC Stevenson, R K Neupane and D Grzywacz (eds). Summary proceedings of a NARC-ICRISAT-NRI work shop, 17-18 November 2004, Kathmandu, Nepal.

Upadhyaya, H.D., and **Gowda, C.L.L.** 2004. Conserving genetic resources and enhancing their use in crop improvement. In Cool Season Legume Crops Germplasm Committee Meeting ASA-CSSA-SSSA International Annual Meeting Seattle, Washington, USA, October 31-November 4, 2004.

Upadhyaya. H.D., and **Gowda, C.L.L.**. 2005. Core collection approach to enhance germplasm utilization in chickpea (C*icer arietinum* L.) and pigeonpea [C*ajanus cajan* (L.) Millsp.]. Invited paper presented in 4th International Food Legumes Research Conference – Food Legumes for Nutritional Security and Sustainable Agriculture. 18-22 October 2005, New Delhi. In Abstracts (ed. M.C. Kharkwal) p. 92.

Upadhyaya. H.D., and **Gowda, C.L.L.**. 2005. Establishment of the Information Sharing Mechanism for Monitoring the Implementation of Global Plan of Action (GPA Programme. Invited paper presented in the First National Stakeholders Workshop of FAO sponsored project on Global Plan of Action. 24-25 Jan. 2005, NBPGR New Delhi, India.

Upadhyaya, H.D., and **Gowda, C.L.L.** 2005. Using core and mini core approaches to identify germplasm: adding values to the working germplasm collection. In ASA-CSSA-SSSA International Annual Meeting Salt Lake City, USA, November 6-10, 2005. http://crops.confex.com/crops/2005am/techprogram/p7129. HTM.

Upadhyaya, H.D., Baum, M., Buhariwalla, H.K., Udupa, S.M., Furman, B.J., Dwivedi, S.L., Hoisington, D.H., Gaur, P.M., Chandra, S., Gowda, C.L.L., Crouch, J.H., and **Valkoun J**. 2005. Mining Chickpea Composite Collection for Allelic Variation. Paper presented in Generation Challenge program Review meeting, 22-26 August 2005, Chennai, India.

Upadhyaya, H.D., Gowda, C.L.L., Buhariwalla, H.K., and **Crouch, J.H.**. 2005. Identifying useful germplasm for crop improvement using core, mini core and molecular marker approaches. p 66 in the Abstracts, International Symposium on Genomic-Based Plant Germplasm Research. 25-28 April 2005, Beijing, China.

Upadhyaya, H.D., Pundir, R.P.S., and **Gowda, C.L.L.**. 2005. Genetic Resources diversity of finger millet – a global perspective. Paper presented in the DFID-Crop Protection Program Project R 8445 "Facilitating the promotion of improved and blast resistant finger millet varieties to enhance production" Stakeholder Workshop, 13-14 Sep. 2005, Nairobi, Kenya.

Upadhyaya, H.D., Pundir, R.P.S., Sube Singh, and **Gowda, C.L.L**. 2005. How to maintain purity and viability in peanut germplasm In Final Review Workshop of ACIAR peanut project ASEM/ 2001/055, Lae, PNG, October 18-19, 2005. Abstract p 13.

Upadhyaya, H.D. 2005. Core and mini core approaches to enhance use of chickpea germplasm collection. 2005. In Cool season Food Legumes meeting, Salt Lake City, USA, November 3-5, 2005.

Vadez, V., Krishnamurthy, L., Gaur, P.M., Upadhyaya, H.D., Hoisington D, and **Varshney, R.K.**. 2006. Genetic variability for salinity tolerance in chickpea. In 3rd International Conference on Legume Genomics and Genetics (9 - 13 April 2006, Brisbane, Queensland, Australia)

Vadez V, Kashiwagi, J, Krishnamurthy, L., Serraj, R., Sharma, K.K., Devi, J., Bhatnagar-Mathur, P., Hoisington, D., Chandra, S., Gaur, P.M., Nigam, S.N., Rupakula, A., Upadhyaya, H.D., Hash, C.T., and **Rizvi, S.M.H.**. 2005. Recent advances in drought research at ICRISAT: Using root traits and rd29a::DREB1A to increase water use and water use efficiency in drought-prone areas. Paper presented in the Interdrought International Conference, 24-28 Sep. 2005. Rome, Italy.

van der Maesen, L.J.G., Pundir, R.P.S., and **Upadhyaya, H.D..** 2005. Use made of wild legume relatives in breeding. Invited paper presented in 4th International Food Legumes Research Conference – Food Legumes for Nutritional Security and Sustainable Agriculture. 18-22 October 2005, New Delhi. In Abstracts (ed. M.C. Kharkwal) p. 29.

Waliyar, F., Kumar, P.L., and **Reddy, S.V.** 2005. Integrated management of preharvest aflatoxin contamination in groundnut. In Proceedings IPM Research at ICRISAT: Present Status and Future Priorities. ICRISAT, Patancheru 502 324, India. p33-40

Waliyar, F., Kumar, P.L. and Reddy, S.V. 2005. Integrated management of preharvest aflatoxin contamination in groundnut. In Proceedings IPM Research at ICRISAT: Present Status and Future Priorities. ICRISAT, Patancheru 502 324, India. p33-40

Waliyar, F., Natre, B.R., Traore, A., Biarra, B., Kodio, O. and Kumar, P.L. 2005. Pre- and Post-harvest management of aflatoxin contamination in groundnut in West and Central Africa. In abstracts of a conference on *Reducing Impact of Mycotoxins in Tropical Agriculture with Emphasis on Health and Trade in Africa*. Accra, Ghana. International Institute of Tropical Agriculture and Myco-Globe, pp20-21

Waliyar, F., Reddy, S.V., Reddy, T.Y., Subramanyam, K., Craufurd, P.Q., Wheeler, T.R., Rama Devi, K., Thakur, R.P., Nigam, S.N., Upadhyaya, H.D., and **Desai, S.** 2005. Management of aflatoxins in groundnut in southern India. Pages 106-108 *In* Summary Proceedings of International Peanut Conference 2005 "Prospects and Emerging Opportunities for Peanut Quality and Utilization Technology", January 9-15 2005, Kasetsart University, Bangkok, Thailand (Abstract).

Waliyar, F., Siambi, M., Jones, R., Reddy, S.V., Chibonga, D., Kumar, P.L., Denloye, S. and Chinoko, Y. 2005. Instiutionalization of aflatoxin testing facility in Africa. In abstracts of a conference on *Reducing Impact of Mycotoxins in Tropical Agriculture with Emphasis on Health and Trade in Africa*. Accra, Ghana. International Institute of Tropical Agriculture and Myco-Globe, pp38.

Journal articles

Aruna, R., Manohar Rao, D., Reddy, L.J., Upadhyaya, H.D., and **Sharma, H.C.** 2005. Inheritance of trichomes and resistance to pod borer (*Helicoverpa armigera*) and their association in interspecific crosses between cultivated pigeonpea (C*ajanus cajan*) and its wild relative C. *scarabaeoides*. *Euphytica* 145: 247-257.

Basandrai, A.K., Pande, S., Kishore, G.K., Crouch, J.H. and **Basandrai, D.** 2005 Cultural, morphological and pathogenic variation in Indian isolates of *Ascochyta rabiei*, the chickpea blight pathogen. *The Plant Pathology Journal* (*in press*).

Bidinger, F.R., Serraj, R., Rizvi, S.M.H., Howarth, C., Yadav, R.S., and Hash, C.T. **2005.** Field evaluation of drought tolerance QTL effects on phenotype and adaptation in pearl millet [*Pennisetum glaucum* (L.) R. Br.] topcross hybrids. Field Crops Research 94:14-32.

Buhariwalla, H.K., Srilakshmi, P., Kannan, S., Kanchi, R.S., Chandra, S., Satyaprasad, K., Waliyar, F., Thakur, R.P., and **Crouch, J.H.** 2005. AFLP analysis of Trichoderma spp. from India compared with sequence and morphological-based diagnostics. Journal of Phytopathology, 153 (7-8) p389-400

Buhariwalla, H.K., Jayashree, B., Eshwar, K., and **Crouch, J.H.** 2005. Development of ESTs from chickpea roots and their use in diversity analysis of the C*icer* genus. BMC Plant Biology (Open-access), 2005, 5:16. http://www.biomedcentral.com/1471-2229/5/16

Buhariwalla, H.K., Jarret, R.L., Jayashree, B., Eshwar, K., Crouch, J.H., and **Ortiz, R.** 2005. Isolation and characterization of microsatellite markers from *M.balbisiana*. Mol. Ecol. Notes, 5, 327-330.

Dwivedi, S.L., Blair, M., Upadhyaya, H.D., Serraj, R., Balaji, J., Buhariwala, H.K., Ortiz, R., and **Crouch, J.H.,** 2005. Using genomics to exploit grain-legume biodiversity in crop improvement. Plant Breeding Review 26:171-358.

Folkertsma, R.T., Rattunde, H.F.W., Chandra, S., Soma Raju, G., and **Hash, C.T.** 2005. The pattern of genetic diversity of Guinea-race *Sorghum bicolor* (L.) Moench landraces as revealed with SSR markers. Theoretical and Applied Genetics 111:399-409.

Gowda, C.L.L., and **Upadhyaya, H.D.**. 2005. International crop germplasm exchange at ICRISAT. Indian J. Plant Genetic Resources 18:25.

Gowda, CLL, Ramesh, S, Chandra, S., and **Upadhyaya, H.D**. 2005. Genetic basis of pod borer (*Helicoverpa armigera*) resistance and grain yield in desi and kabuli chickpea (C*icer arietinum* Linn.). Euphytica 145:199-214.

Grirjashankar, V., Sharma, H.C., Sharma, K.K., Swathisree, V., Sivarama Prasad, L., Bhat, B.V., Royer, M., Secundo, B.S., Narasu, L.M., Altosaar, I., and **Seetharama, N.** 2005. Development of transgenic sorghum for insect resistance against the spotted stem borer (C*hilo partellus*). Plant Cell Reports 24: 513-522.

Jayashree, B., Buhariwalla, H.K., Shinde, S., and **Crouch, J.H.** 2005. A legume genomics resource: The chickpea root EST database. *Electronic Journal of Biotechnology*, 8:2. (Open-access) http://www.ejbiotechnology.info/content/vol8/issue2/abstract/8/

Jayashree, B., Ferguson, M., Dan Ilut, M., Doyle., and **Crouch, J.H.** 2005. Analysis of genomic sequence from peanut (*Arachis hypogaea*). *Electronic Journal of Biotechnology*, 8: 3. (Open-access) http://www.ejbiotechnology.info/content/vol8/issue3/abstract/3/

Jayashree, B., Rangacharyulu, A.V., Sreedhar, T., and **Chandra, S.** 2005. Design and implementation of an integrated database for genetic and genomic data :ICRIS. In Bioinformatics Computing, Editors D Garg and Amardeep Singh; Allied Publishers Pvt. Ltd. pp. 60 –63.

Kalyani, G., Sonali, S., Reddy, A.S., Reddy, A.G.S., Waliyar, F., , and **S N Nigam.** 2005. Resistance to tobacco streak virus in groundnut, *Arachis hypogaea*. *Journal Of Oilseeds Research* 22(1):105-107.

Kashiwagi, J., Krishnamurthy, L., Upadhyaya, H.D., **Hari Kishana, Chandra, S., Vincent, V.,** and **Serraj, R**. 2005. Genetic variability of drought-avoidance root trait in the mini core germplasm collection of chickpea (C*icer arietinum* L.). Euphytica 146:213-222.

Kiranmai, G., Kumar, P.L., Hema, M., Venkatramana, M., Krishna Prasadji, J., Rao, D.M. and **Sreenivasulu, P.** 2005. Partial characterization of a potyvirus causing bract mosaic of banana in Andhra Pradesh. *Indian Journal of Virology* 16: 7-11.

Kishore, G.K. and **Pande, S**. 2005. Integrated management of late leaf spot and rust diseases of groundnut using leaf extract of *Datura metel* and chlorothalonil. *Australasian Plant Pathology* 34 (2): 261-264.

Kishore, G.K., Pande, S. and **Podile, A.R.** 2005. Management of control of late leaf spot of groundnut (*Arachis hypogaea*) with chlorothalonil-tolerant isolates of *Pseudomonas aeruginosa*. *Plant Pathology* 54 (3): 401-408.

Kishore, G.K., Pande, S. and **Podile, A.R.** 2005. Biological control of late leaf spot of peanut (*Arachis hypogaea* L.) with chitinolytic bacteria. *Phytopathology* 95(10): 1157-1165.

Kishore, G.K., Pande, S. and **Podile, A.R**. 2005. Biological control of collar rot disease with broad-spectrum antifungal bacteria associated with groundnut. C*anadian Journal of Microbiology* 51 (2): 123-132.

Kishore, G.K., Pande, S. and **Podile, A.R**. 2005. Chitin-supplemented foliar application of *Serratia marcescens* GPS 5 improves control of late leaf spot disease of groundnut by activating defense-related enzymes. *Journal of Phytopathology* 153 (3): 169-173.

Kishore, G.K., Pande, S. and **Podile, A.R.** 2005. Phylloplane bacteria increase seedling emergence, growth and yield of field-grown groundnut *(Arachis hypogaea* L.). *Letters in Applied Microbiology* 40 (4): 260-268.

Kishore, G.K., Pande, S., Rao, J.N. and **Podile, A.R**. 2005. *Pseudomonas aeruginosa* inhibits the plant cell wall degrading enzymes of *Sclerotium rolfsii* and reduces the severity of groundnut stem rot. *European Journal of Plant Pathology* 113 (3): 315-320.

Kishore, G.K., Pande, S., Shivram, L., and **Siddique, K.H.M.** 2005. Cultural, morphological, pathogenic and genetic variation in *Botrytis cinerea*, causal agent of gray mold in chickpea. *Australian Journal of Agricultural Research* (*under communication*).

Kranthi, K.R., Jadhav, D.R., Kranthi, S., Wanjari, R.R., Ali, S.S., and **Russel, D.A.** 2002. Insecticide resistance in five major insect pests of cotton in India. C*rop Protection* 6: 449-460.

Kresovich, S., Barbazuk, B., Bedell, J., Borrell, A., Buell, R., Burke, J., Clifton, S., Cordonnier-Pratt, M-M, Cox, S., Dahlberg, J., Erpelding, J., Fulton, T.M., Fulton, B., Fulton, L., Gingle, A., Goff, S., Hash, C.T., Huang, Y., Jordan, D., Klein, P., Klein, R., Magalhaes, J., McCombie, R., Moore, P., Mullet, J.E., Ozias-Akins, P., Paterson, A.H., Porter, K., Pratt, L., Roe, B., Rooney, W., Schnable, P., Stelly, D.M., Tuinstra, M., Ware, D., and **Warek, U.** 2005. **Toward sequencing the** *Sorghum* **genome. A U.S. National Science Foundation-sponsored workshop report. Plant Physiology 138:1898–1902.**

Kumar, P.L., Jones, A.T. and Waliyar, F. 2004. Biology, etiology and management of pigeonpea sterility mosaic disease. *Annual Review of Plant Pathology* 3: 1-24 (Published in 2005)

Kumar, P.L., Jones, A.T., and **Waliyar, F.** 2004. Biology, etiology and management of pigeonpea sterility mosaic disease. *Annual Review of Plant Pathology* 3: 70-100 (Published in 2005)

Kumar, P.L., Latha, T.K., Kulkarni, N.K., Raghavendra, K., Saxena, K.B., Waliyar, F., Rangaswamy, K.T., Muniyappa, V., Sabitha D., and **Jones, A.T.** 2005. Broad-based resistance to pigeonpea sterility mosaic disease in wild relatives of pigeonpea (C*ajanus:* Phaseoleae). *Annals of Applied Biology* 146:371-379.

Mgonja, M.A., Chandra, S., Gwata, E.T., Obilana, A.B., Monyo, E.S., Rohrbach, D.D., Chisi, M., and **Saadan, M.H.** 2005. Improving the efficiencies of national crop breeding programs through region-based approaches: The case of sorghum and pearl millet un southern Africa. Journal of Food Agriculture and Environment 3:124-129

Mallikarjuna, N., Kranthi, K.R., Jadhav, D.R., Kranthi, S., and **Chandra S**. 2004. Influence of foliar chemical compounds on the development of *Spodoptera litura* (Fab.) in interspecific derivatives of groundnut. *Journal of Applied Entomology* 28:(5) 321-328.

Mallikarjuna, N., Pande, S., Jadhav, D.R. Sastri, C. and **Narayan Rao, J**. 2004. Introgression of disease resistance genes from *Arachis kempff-mercadoi* into cultivated groundnut. *Plant Breeding* 123 (6): 573-576.

Mallikarjuna, N., and **Saxena, K.B.** 2005. A new cytoplasmic male-sterility system derived from cultivated pigeonpea cytoplasm. Euphytica 142 (1-2):143-148.

Mallikarjuna, N., Jadhav, D.J., Reddy, M.V., and **Usharani Dutta, T.,** 2005. Introgression of Phytophthora blight disease resistance from C*ajanus platycarpus* into short duration pigeonpeas. Indian J. Genet. Plant Breed 65 (4): 261-264.

Mallikarjuna, N., Maheedhar, G., and **Chandra, S.** 2005. Genetic diversity among *Arachis* species based on RAPDs. IJGPB 65 (1): 5-8.

Nigam, S.N., Chandra, S., Rupa Sridevi, K., Bhukta, M., Wright, G.C., Rachaputi, N.R., Cruickshank, A.W., Reddy, P.V., and **Deshmukh, M.P.** 2005. Efficiency of empirical and trait-based selection for kernel yield in groundnut. Annals of Applied Biology 146:433-439

Pande, S., Siddique, K.H.M., Kishore, G.K., Bayaa, B. Gaur, P.M., Gowda, C.L.L., Bretag, T.W., and **Crouch, J.H.** 2005. Ascochyta blight of chickpea (C*icer arietinum* L.): a review of biology, pathogenicity and disease management. Australian Journal of Agricultural Research 56:317-332.

Pande, S., Stevenson, P., Rao, J.N., Neupane, R.K., Chaudhary, R.N., Grzywacz, D., Bourai, V.A. and **Kishore, G.K**. 2005. Reviving chickpea production in Nepal through integrated crop management, with emphasis on Botrytis gray mold. *Plant Disease* 89 (12): 1252-1262 (*feature article*).

Reddy, L.J., Upadhyaya, H.D., Gowda, C.L.L., and **Sube Singh**. 2005. Development of core collection in pigeonpea [C*ajanus cajan* (L.) Millspaugh] using geographic and qualitative morphological descriptors. Genetic Res. and Crop Evolution 52:1049-1056.

Serraj, R., Hash, C.T., Rizvi, S.M.H., Sharma, A., Yadav, R.S., and **Bidinger, F.R.** 2005. Recent advances in marker-assisted selection for drought tolerance in pearl millet. Plant Production Science 8(3, special issue: Proceedings of the Fifth Asia Crop Science Conference):334–337.

Sharma, H.C., Pampapathy, G., and **Kumar, R.** 2005. Standardization of cage technique to screen chickpeas for resistance to *Helicoverpa armigera* (Lepidoptera: Noctuidae) in greenhouse and field conditions. *Journal of Economic Entomology* 98: 210-216.

Sharma, H.C., Pampapathy, G., Dhillon, M.K., and **Ridsdill-Smith, T.J**. 2005. Detached leaf assay to screen for host plant resistance to *Helicoverpa armigera*. *Journal of Economic Entomology* 98: 568-576.

Sharma, H.C., Pampapathy, G., Lanka, S.K., and **Ridsdill-Smith, T.J.** 2005. Antibiosis mechanism of resistance to legume pod borer, *Helicoverpa armigera* in wild relatives of chickpea. *Euphytica* 142: 107-117.

Sharma, K.K., Bhatnagar-Mathur, P. and **Thorpe, T.A.** 2005. Genetic transformation technology: status and problems. In Vitro Cellular & Developmental Biology-Plant, 41: 102-112.

Shukla, S., Arora, R., and **Sharma, H.C.** 2005. Biological activity of soybean trypsin inhibitor and plant lectins against cotton bollworm/legume pod borer, *Helicoverpa armigera* (Noctuidae: Lepidoptera). Plant Biotechnology 22: 1-6.

Sonali Shukla.,, Kalyani, G., Kulkarni, N., Waliyar, F., and Nigam, S.N. 2005. Mechanism of transmission of tobacco streak virus by *Scirtothrips dorsalis*, *Frankliniella schultzei* and *Megalurothrips usitatus* in groundnut. *Journal of Oilseeds Research*, 22(1):215-217.

Upadhyaya, H.D., Mallikarjuna Swamy, B.P., Kenchana Goudar, P.V., Kullaiswamy, B.Y., and **Sube Singh**. 2005. Identification of diverse accessions of groundnut through multienvironment evaluation of core collection for Asia. Field Crops Research 93: 293-299.

Upadhyaya, H.D., Pundir, R.P.S., Gowda, C.L.L., Reddy, K.N., and **Sube Singh**. 2005. Geographical pattern of diversity for qualitative and quantitative traits in pigeonpea germplasm collection. Plant Genetic Resources: Characterization and Utilization 3: 331-352.

Upadhyaya, H.D. 2005. Variability for drought resistance related traits in the mini core collection of peanut. Crop Science 45: 1432-1440.

Waliyar, F., Traore, D., Fatondji, D., and Ntare, B.R. 2003. Effect of irrigation interval, planting date, and cultivar on *Aspergillus flavus* and aflatoxin contamination of peanut in a sandy soil of Niger. Peanut Science 30: 79-84. (Published in 2005)

Miscellaneous

Abstracts/Bulletins/Posters/News letter articles/etc.

Basandrai, A.K., Basandrai, D., Pande, S., Gaur, P.M. and **Thakur, H.L.** 2005. Identification of stable sources of resistance against Ascochyta blight in chickpea. Page 44 *in* Abstracts of Centenary Symposium on Plant Pathology, April 7-8, 2005, Central Potato Research Institute, Shimla 171001, HP, India

Blümmel, M., Ramakrishna Reddy, Ch., Ravi, D., Nigam, S.N., and **Upadhyaya, H.D**. 2005. Food-fodder traits in groundnut. International Arachis Newsletter 25:52-54

Blümmel, M., Vellaikumar, S., Devulapalli, R., Nigam, S.N., **Upadhyaya, H.D.,** and **Khan, A.**. 2005. Preliminary observations on livestock productivity in sheep fed exclusively on haums from eleven cultivars of groundnut. International Arachis Newsletter 25:54-57.

Chhabra AK, Rai KN, Khairwal IS, Sivaramakrishnan S and **Hash CT.** 2001 (published 2005). Effect of pollen and DNA source on RFLP pattern of mitochondrial DNA in pearl millet. International Sorghum and Millets Newsletter 42:93–96.

Coyne, C.J., Baum, M., Sharma, P.C., Gaur, P.M., Muehlbauer, F.J., McPhee, K.E., Timmerman-Vaughan, G.M., Pilet-Nayel, M.L., Brown, A.F., and **McGee, R.J.** 2005. Application of molecular markers in food legumes breeding. Page 17 *in* Abstracts of Fourth International Food Legumes Research Conference, 18-22 October 2005, Indian Agricultural Research Institute, New Delhi, India.

Coyne, C.J., Sharp-Vincent, T., Cashman, M.J, Watt, C.A., Chen, W., Muehlbauer, F.J., and **Mallikarjuna, N.** 2005. A method for germinating perennial C*icer* species. International Chickpea and Pigeonpea Newsletter 12: 28-29.

Davenport, G., Chandra, S., Akintunde, A., Hoisington, D., and **Jayashree, B**. LIMS web applications - sample tracking. Poster presented at the Generation Challenge Program Annual Research Meeting, Rome, 29th Sep-1st October 2005.

Devadas, R., Mallikarjuna, N., Rao, T.N., and **Pandey, S.,** 2005. Important traits in annual wild Cicer species for chickpea (C*icer arietinum* L.) improvement. (poster.) IFLRC, New Delhi.

Devos, K.M., Sivaramkrishnan, S., Zerbini, E. and **Hash, C.T.** QTLs controlling stover quality traits in pearl millet (*Pennisetum glaucum* L. R. Br.) and opportunities for marker assisted selection. Page 194 *in* Abstracts, International Conference on "Plant genomics and biotechnology: Challenges & opportunities", October 26-28, Indira Gandhi Agricultural University, Raipur, Chhattisgarh, India.

Folkertsma, R.T., Mouli, R.C., Bidinger, F.R., Mahalakshmi, V., and **Hash, C.T.** 2005. Association mapping of stay-green in sorghum. Final Program and Abstract Book, InterDrought-II. The 2nd International Conference on Integrated Approaches to Sustain and Improve Plant Production Under Drought Stress, September 24 to 28, 2005, University of Rome "La Sapienza", Rome, Italy. P. 8.13.

Gale MD, Devos KM, Zhu J, Allouis S, Couchman MS, Liu H, Pittaway TS, Qi XQ, Kolesnikova-Allen M and **Hash CT.** 2001 (published 2005). New molecular marker technologies for pearl millet improvement. International Sorghum and Millets Newsletter 42:16–22.

Gaur, P.M., and **Gowda, C.L.L.** 2005. Trends in world chickpea production, research and development. Pages 8-15 *in* Proceedings of Focus 2005: Chickpea in the farming systems, Sept 21-23, 2005, Goondiwindi, Qld, Australia (Knights, T. and Merrill, R., ed.). Toowoomba, Australia: Pulse Australia.

Gaur, P.M., Pande, S., Sharma, H.C., Gowda, C.LL. Sharma, K.K. Crouch, J.H. and **Vadez, V. 2005.** Genetic enhancement of stress tolerance in chickpea: present status and future prospects. Page 197 *in* Abstracts of International Conference on Sustainable Crop Production in Stress Environments: Management and Genetics Options, 9-12 February 2005, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, MP, India.

Gaur, P.M., Pratibha, R., Pande, S., Sharma, H.C., Vadez, V., Kashiwagi, J. and **Hoisington, D.A.** 2005. Molecular mapping of resistance to biotic and abiotic stresses in chickpea. Page 38 *in* Abstracts of International Conference on Plant Genomics and Biotechnology, 26-28 October 2005, Indira Gandhi Agricultural University, Raipur, Chhattisgargh, India.

Gulia, S.K., Hash, C.T., Thakur, R.P., Breese, W.A., and **Sangwan, R.S..** 2005. Mapping new QTLs for improvement of downy mildew [*Sclerospora graminicola* (Sacc.) J. Schroet.] in pearl millet. Pages 279–280 *in* Abstracts, International Conference on Sustainable Crop Production in Stress Environments: Management and Genetic Options, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, (M.P.), India, February 9–12, 2005.

Hash, C.T., and **Witcombe, J.R.** 2001 (published 2005). Pearl millet molecular marker research. International Sorghum and Millets Newsletter 42:8–15.

Hash, C.T. 2005. Opportunities for application of molecular markers for sustainable crop production in stress environments: Sorghum and pearl millet. Page 13 *in* Abstracts, International Conference on Sustainable Crop Production in Stress Environments: Management and Genetic Options, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.), India, February 9–12, 2005.

Hash, C.T., Folkertsma, R.T., Ramu, P., Mouli, R.C., Venkateswara Rao, S., Somaraju, G., Chanda, A.K., Sivaramakrishnan, S., Mahalakshmi, V. and **Bidinger, F.R.** 2005. Slow progress in marker-assisted backcrossing of stay-green in sorghum. Final Program and Abstract Book, InterDrought-II. The 2nd International Conference on Integrated Approaches to Sustain and Improve Plant Production Under Drought Stress, September 24 to 28, 2005, University of Rome "La Sapienza", Rome, Italy. L. 8.07.

Hash, C.T., Thakur, R.P., and **Azhaguvel, P.** 2005. Mapping-directed conventional backcrossing for improved pearl millet downy mildew resistance. Agronomy Abstracts 163-6.

Hoisington, D., Upadhyaya, H.D., Dwivedi, S.L., Baum, M., Udupa, S.M., Furman, B.J., Chandra, S., Eshwar, K., Gowda, C.L.L., and **Singh, S**. 2006. Genotypic and phenotypic variation in the global collection of chickpea (C*icer arietinum* L.). Accepted for presentation in the 3rd International Conference on Legume Genomics & Genetics, Brisbane, Australia, April 9-13 2006.

Hoisington, D.A., Varshney, R., and **Hash, C.T.** 2005. Predicting phenotypes from genotypes – the application of comparative and functional genomics. Pages 9-10 *in* Abstracts, International Conference on "Plant genomics and biotechnology: Challenges & opportunities", October 26-28, Indira Gandhi Agricultural University, Raipur, Chhattisgarh, India.

Kashiwagi, J., Krishnamurthy, L., Upadhyaya, H.D., Chandra, S. and **Gaur, P.M.** 2005. Genetic control of drought avoidance root traits in chickpea (C*icer arietinum* L.). Page 91 *in* Abstracts of Fourth International Food Legumes Research Conference, 18-22 October 2005, Indian Agricultural Research Institute, New Delhi, India.

Kumar, P.L., 2004. ICP7035: A dual-purpose pigeonpea variety with broad-based resistance to sterility mosaic disease. Information Broacher for Pigeonpea Scientists Meeting at ICRISAT, 9 December 2004, ICRISAT, Patancheru, India. 1pp.

Kumar, P.L. 2004. Virology @ ICRISAT. In Vision-2010, colloquium cum walk-through exhibition, 14 Decemb er 04, ICRISAT, Patancheru 502 324, AP, India.

***Kumar, P.L.** and **Waliyar, F.** 2005*. Advances in diagnosis of eriophyid-mite transmitted viruses and virus-like agents of undefined etiology, with a particular reference to pigeonpea sterility mosaic disease. In Abstracts 2nd Global Conference on Plant Health - Global Wealth, Rajasthan College of Agriculture and Technology, Udaipur, India.

***Kumar, P.L., Latha, T.K.S., Chauhan, V.B., Aparna, V and Waliyar. F.** 2005*. Studies on Pigeonpea sterility mosaic virus isolates in India. In Abstracts 2nd Asian Congress of Plant Pathology 25-28 June 2005, National University of Singapore, Singapore. P85-86

Kumar, P.L., Narayana, Y.D., Muniyappa, V., Rangaswamy, K.T., Jones, A.T., Waliyar, F., and **Kimmins, F.** 2005. Epidemiology and Management of pigeonpea sterility mosaic virus. In Abstract *IX International Plant Virus Epidemiology Symposium*. April 4-7, Lima, Peru. p52.

Kumar, P.L., Saxena, K.B. and Waliyar, F. 2005. Boon to Sterility Mosaic Resistance. *SATrends 53 (e-newsletter on http://www.icrisat.org)*.

Lulsdorf, M., Mallikarjuna, N., Clarke, H., and **Tar'an, B.,** 2005. Finding Solutions for Interspecific Hybridization Problems in Chickpea. (Abstr.) IFLRC, New Delhi.

Mallikarjuna, N. 2005. Breakthrough in chickpea research. SATrends, August, 2005.

Mallikarjuna, N., and **Jadhav, D.** 2005. Hybrids between *Arachis hypogaea* and *A. kretchmeri* from section *Procumbentes*. (Abstr.) American Peanut Research Education Society, Florida, USA.

Mallikarjuna, N., Jadhav, D., Clarke, H., Coyne, C., and **Muehlbauer F.J.,** 2005. Induction of androgenesis as a consequence of wide crossing in chickpea. International Chickpea and Pigeonpea Newsletter 12: 12-15.

Mallikarjuna, N., Muehlbauer, F.J., Saxena, K.B., Hoisington, D., Clarke, H., and **Coyne, C.,** 2005. Unlocking genetic potential of wild species for legume crop improvement. (Abstr.) IFLRC, New Delhi.

Mallikarjuna, N., Sharma, H.C., Jadhav, D., Pampapathy, G., and Mei, Y., Sharma, K.K., Anjaiah, V., LI, S.-L., Tao, H.-T., Ren, Y. and Yu, S.-l. 2005. An effective method for cloning of partial MADS-Box genes related to flower development in groundnut. International Arachis Newsletter, 25: 30-32.

Mukhopadhyay R, Hash CT, Bhasker Raj AG and **Kavi Kishor PB.** 2005. Assessing opportunities to map pearl millet tolerance to salinity during germination and early seedling growth. Pages 401–402 *in* Abstracts, International Conference on Sustainable Crop Production in Stress Environments: Management and Genetic Options, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.), India, February 9–12, 2005.

Mukhopadhyay R, Hash CT, Bhasker Raj AG and **Kavi Kishor PB.** 2005. Assessment of opportunities to map pearl millet tolerance to salinity during germination and early seedling growth. International Sorghum and Millets Newsletter 46: 117–120.

Nepolean, T., Blummel, M., Rajaram, V., Senthilvel, S., Yadav, R.S., Howarth, C.J.,

Nepolean, T., Blummel, M., Rajaram, V., Senthilvel, S., Yadav, R.S., Howarth, C.J., Devos, K.M., Sivaramkrishnan, S., Zerbini, E. and Hash, C.T. QTLs controlling stover quality traits in pearl millet (*Pennisetum glaucum* L. R. Br.) and opportunities for marker assisted selection. Page 194 *in* Abstracts, International Conference on "Plant genomics and biotechnology: Challenges & opportunities", October 26-28, Indira Gandhi Agricultural University, Raipur, Chhattisgarh, India.

Ngugi, K., Muthamia, Z., and **Folkertsma, R**. 2005. Molecular marker-assisted selection of three intractable traits of maize and sorghum inKenyan genetic backgrounds: myth or reality? Paper presented at the Conference on "Biotechnology, Breeding and Seed Systems for African Crops" 24-27 January 2005, Nairobi, Kenya.

Ortiz, R., Ban T., Bandyopadhyay, R., Bergvinson, D., Hell, K., Jeffers, D., Menkir, A., Murakami, J., Kumar, P.L., Nigam, S.N., Upadhyaya, H.D., and **Waliyar. F.** 2005. CGIAR research-for-development program on mycotoxins. In abstracts of a conference on *Reducing Impact of Mycotoxins in Tropical Agriculture with Emphasis on Health and Trade in Africa*. Accra, Ghana. International Institute of Tropical Agriculture and Myco-Globe, pp44-45.

Pande, S., Upadhyaya, H.D., Naryana Rao, J., Lakshmi Reddy, P., and **Parthasarathy Rao, P.**. 2005. Promotion of Integrated Disease Management for ICGV 91114, a dual-purpose, early maturing groundnut variety for rainfed areas. Information Bulletin No. 68. Patancheru, 502324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 28 pp. ISBN No. 92-9066-480-0.

Pande, S. Kishore, G.K., Gaur, P.M., Basandrai, A.K. and **Kannan, S.** 2005. Variation in chickpea fungal pathogens: challenging the resistance breeding. Pages 40-41 *in* Abstracts of International Conference on Plant Genomics and Biotechnology: Challenges and Opportunities, 26-28 October 2005, Indira Gandhi Agricultural University, Raipur, Chhattisgargh, India.

Pande, S., Basandrai, A.K., Kishore, G.K., Gaur, P.M., Kannan, S., Rao, J.N., Crouch, J. H., Siddique, K.H.M., and **Gowda, C.L.L.** 2005. Morphological, pathogenic and genetic variability in Indian isolates of *Ascochyta rabiei* of chickpea. 15th Australasian Plant Pathology Society Conference, Melbourne, Australia.

Pande, S., Basandrai, A.K., Kishore, G.K., Gaur, P.M., Kannan, S., Rao, J.N. and **Crouch, J.H.** 2005. Variability in Indian isolates of Ascochyta rabiei, the blight pathogen of chickpea. Pages 302-303 *in* Abstracts of Fourth International Food Legumes Research Conference, 18-22 October 2005, Indian Agricultural Research Institute, New Delhi, India.

Pande, S., Kishore, G.K. and **Rao, J.N.** 2005. Marigold - A diagnostic tool for BGM forecasting and management in chickpea. *International Chickpea and Pigeonpea Newsletter* 12:2-3.

Pratibha, R., Gaur, P.M., Crouch, J.H., Pande, S., Kishore, G. K., Kannan, S. and **Katiyar, S.K.** 2005. Construction of an intraspecific linkage map of chickpea (C*icer arietinum* L.) based on SSR markers and identification of QTLs for Ascochyta Blight resistance. Page 157 *in* Abstracts of International Conference on Plant Genomics and Biotechnology: Challenges and Opportunities, 26-28 October 2005, Indira Gandhi Agricultural University, Raipur, Chhattisgargh, India.

Ramu, P., Hash, C.T., Jayashree B., Chandra, S., Rekha B., Prasad, P.V.N.S., Mahalakshmi, V., and **Crouch, J.H.** 2005. Development and application of EST-SSR markers in sorghum for comparative genome mapping with rice. Pages 404-405 *in* Abstracts, International Conference on Sustainable Crop Production in Stress Environments: Management and Genetic Options, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.), India, February 9-12, 2005.

Rangaswamy, K.T., Byregowda, M., Kumar, P.L., Saxena, K.B. and Waliyar, F. 2005. Release proposal of the sterility mosaic disease resistant pigeonpea variety ICP7035 as 'Teja'. University of Agriculture Sciences Bangalore and ICRISAT, Patancheru 502 324, India. 27pp. Release proposal

Rangaswamy, K.T., Kumar, P.L., Prameela, P.A., Raghavendra, N. and **Shankarappa, S.** 2004. Pigeonpea sterility mosaic disease resistant variety ICP 7035 and crop management (In Kannada Language). Information Broacher. University of Agriculture Sciences, GKVK, Hebal, Karnataka 560065, India. 4 pp.

Rangaswamy, K.T., Muniyappa, V., Kumar, P.L., Saxena, K.B., Byregowda, M., Raghavendra, N., Pandurangaiah, K., Kumar, R.V., Waliyar, F. and Jones, A.T. 2005. ICP 7035 - A sterility mosaic resistant vegetable and grain purpose pigeonpea variety for Karnataka state of India. *International Chickpea and Pigeonpea Newsletter* 12: 42-44.

Reddy, A.S., Kumar, P.L. and Waliyar, F. 2005. Rate of Transmission of *Indian peanut clump virus* (IPCV) to Groundnut by Mechanical Inoculation. *International Arachis Newsletter* 25: 37-39.

Rizvi, R., Serraj, R.,, Hash, C.T., and **Vadez V.** 2005. Screening for P acquisition from rock phosphate in pearl millet. Page 255 *in* Abstracts, International Conference on Sustainable Crop Production in Stress Environments: Management and Genetic Options, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.), India, February 9–12, 2005.

Rizvi, S.M.H., Serraj, R., Bidinger, F.R., Yadav, O.P., and **Hash, C.T.** 2005. Validation of a QTL for terminal drought tolerance in pearl millet. Final Program and Abstract Book, InterDrought-II. The 2nd International Conference on Integrated Approaches to Sustain and Improve Plant Production Under Drought Stress, September 24 to 28, 2005, University of Rome "La Sapienza", Rome, Italy. P. 2.80.

Rizvi, S.M.H., Murty, T.S., Vadez, V. and **Hash, C.T.** Leaf gas exchange and ABA response to soil drying in pearl millet (*Pennisetum glaucum* (L.) R.Br.). Page 168 *in* Abstracts, International Conference on "Plant genomics and biotechnology: Challenges & opportunities", October 26-28, Indira Gandhi Agricultural University, Raipur, Chhattisgarh, India.

Rizvi, S.M.H., Serraj, R., Bidinger, F.R., Sharma, A., Nijhawan, D.C., Yadav, R.S., Bhasker Raj, A.G., Howarth, C.J., Vadez, V., and **Hash, C.T.** 2005. Field assessment of backcross-derived hybrids validates a major pearl millet drought tolerance QTL. Pages 219–220 *in* Abstracts, International Conference on Sustainable Crop

Production in Stress Environments: Management and Genetic Options, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, (M.P.), India, February 9–12, 2005.

Rizvi, S.M.H., Serraj, R., Bidinger, F.R., Yadav, O.P. and **Hash, C.T.** 2005. Validation of a QTL for terminal drought tolerance in pearl millet. Final Program and Abstract Book, InterDrought-II. The 2nd International Conference on Integrated Approaches to Sustain and Improve Plant Production Under Drought Stress, September 24 to 28, 2005, University of Rome "La Sapienza", Rome, Italy. P. 2.80.

Sathish Kumar P, Hash CT, Yadav RS, Bidinger FR, Bhasker Raj AG, Howarth CJ, Waterman E, Devos KM and **Muralidharan V.** 2005. Marker-assisted backcrossing (MABC) to improve terminal drought tolerance in pearl millet hybrid seed parent ICMA/B 841. Pages 405–406 *in* Abstracts, International Conference on Sustainable Crop Production in Stress Environments: Management and Genetic Options, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, (M.P.), India, February 9–12, 2005.

Saxena, K.B. and **Kumar, P.L**. 2005. SMD resistant pigeonpea variety (in Kanada). *Krisika Bhandu*, March 2005.

Saxena, K.B., Kumar, R.V., Dalvi, V.A., Mallikarjuna, N., Gowda, C.L.L., Singh, B.B., Pandey, L.B. Venkteshwar, S., Paralkar, R.M. Shiying, B., and **Xuxiao, Z**. 2005. Hybrid breeding in grain legumes. Invited lead presentation. IFLRC, New Delhi, India.

Serraj, R., Hash, C.T., Crouch, J.H., Rizvi, M.S.H. (*sic*), and **Lagoda, P.J.** 2005. Genetic and management options to enhance drought resistance and water use efficiency in dryland agriculture. Final Program and Abstract Book, InterDrought-II. The 2nd International Conference on Integrated Approaches to Sustain and Improve Plant Production Under Drought Stress, September 24 to 28, 2005, University of Rome "La Sapienza", Rome, Italy. L. 7.03.

Shahal Abbo and **Nalini Mallikarjuna**, 2005. Distant hybridization in food legumes. Invited lead presentation. IFLRC, New Delhi.

Sharma, H.C., Reddy, B.V.S., Dhillon. M.K., Venkateswaran, K.,, Singh, B.U., Pampapathy, G., Folkertsma, R.T., Hash, C.T., and **Sharma, K.K>.** 2006. Host plant resistance to insects in sorghum: Present status and need for future research. International Sorghum and Millets Newsletter 46: 36–43.

Sharma, H.C., Van Driesche, Arora, R., and **Sharma, K.K**. 2005. Biological activity of lectins from grain legumes and garlic against the legume pod borer, *Helicoverpa armigera*. International Chickpea and Pigeonpea Newsletter 12: 50-53.

Sirisha, P.K., Prasad, R.G., Hussain, A.S., Elango, P., Davenport, G., McLaren, G., Crossa, J., Jayashree, B., Hoisington, D., and **Chandra, S. 2005**. iMAS: An integrated decision support system for marker-assisted plant breeding.Poster presented at the Generation

Sreedhar, T., Leeladevi, Y., Prasanth, V.P., Rajalakshmi, R., Elango, P., Anil Kumar, K, Prasad, P.V.N.S., Chandra, S., Jayashree, B., and Hoisington, D. 2005. Integrating genotyping workflow and databases: an update on Bioinformatics at ICRISAT. Poster presented at the Generation Challenge Program Annual Research Meeting, Rome, 29th Sep-1st October 2005.

Upadhyaya, H.D., and **Pundir, R.P.S**. 2005. ICRISAT Genebank in Service to the Indo-Gangetic Region. ICRISAT Governing Board Meeting at New Delhi, March 2005.

Upadhyaya, H.D., Baum, M., Dwivedi, S.L., Udupa, S.M., Buhariwalla, H.K., Furman, B.J., Chandra, S., Hoisington, D., Crouch, J.H., Gowda, C.L.L., and **Sube Singh**. 2005. Mining allelic variation associated with beneficial traits in composite collection of chickpea (C*icer arietinum* L.). Presented at Generation Challenge Program meeting in Rome, September 25-Ocober 1, 2005.

Upadhyaya, H.D., Bhattacharjee, R., Bertioli, D., Hoisington, D., Pundir, R.P.S., Valls, J.F.M., Chandra, S., Sube Singh, Moretzsohn, M.C., Leal Bertioli, S., and **Guimaraes, P**. 2005. Genotyping Groundnut (*Arachis hypogaea*) Composite Collection Using SSR Markers. Presented at Generation Challenge Program meeting in Rome, September 25-Ocober 1, 2005.

Upadhyaya, H.D, Hildebrand, G.H., Nigam, S.N., and **Yellaiah, N**. 2005. Groundnut cultivar Nyanda (ICGV 93437) released in Zimbabwe. International Arachis Newsletter 25: 22-24.

Upadhyaya, H.D., Nigam, S.N.,, Reddy A.G.S., and **Yellaiah, N**. 2004. Early-maturing, large-seeded, and high-yielding groundnut varieties ICGV 96466, ICGV 96468, and ICGV 96469. International Arachis Newsletter 25:19-21

Upadhyaya, H.D., , **Pundir, R.P.S.,, Hash, C.T., Hoisington, D., Chandra, S., Gowda, C.L.L., Sube Singh.,** and **Gopal Reddy, V**. 2005. Genotyping Finger Millet Germplasm – Developing Composite Collection. Presented at Generation Challenge Program meeting in Rome, September 25-Ocober 1, 2005.

Upadhyaya, H.D., Hoisington, D. 2005. Wide crosses for *Helicoverpa* resistance with reference to pigeonpea and chickpea. (poster.) IFLRC, New Delhi.

Vadez, V., Kashiwagi, J., Krishnamurty, L., Serraj, R., Sharma, K.K., Devi, J., Bhatnagar-Mathur, P., Hoisington, D., Chandra, S., Gaur, P.M., Nigam, S.N., Rupakula, A., Upadhyaya, H.D., Hash, C.T., and **Rizvi, S.M.H.** 2005. Recent advances in drought research at ICRISAT: Using root traits and rd29a:DREB1A to increase water use and water use efficiency in drought-prone areas. Final Program and Abstract Book, InterDrought-II. The 2nd International Conference on Integrated Approaches to Sustain and Improve Plant Production Under Drought Stress, September 24 to 28, 2005, University of Rome "La Sapienza", Rome, Italy. P. 5.88.

Velu, G., Rai, K.N., Muralidharan, V., Kulkarni, V.N., Raveendran, T.S., Longvah, T., and Hash, C.T. 2005. Prospects of genetic enhancement of iron and zinc in pear millet. Agronomy Abstracts 50-6

Waliyar, F., Reddy, S.V., Reddy, T.Y., Subramanyam, K., Craufurd, P.Q., Wheeler T.R., Rama Devi, K., Thakur, R.P., Nigam, S.N., Upadhyaya, H.D., and **Desai, S**. 2005. Management of aflatoxins in groundnut in southern India. Poster presented in the International Peanut Conference 2005. Bangkok, Thailand 10-12 January 2005.

Waliyar, F., and Kumar, P.L. 2005. Towards Safe-to-Eat Maize! ***SATrends 52 (e-newsletter on*** *http://www.icrisat.org*) **. Electronic newsletter**

Waliyar, F., Kumar, P.L. and **Reddy, S.V. 2004.** Managing aflatoxins in maize - information leaflet (In English and Telugu). International Crops Research Institute for the Semi-Arid Tropics, Patancheru 50234, AP, India.

Waliyar, F., Natre, B.R., Traore, A., Biarra, B., Kodio, O. and **Kumar, P.L**. 2005. Pre- and Post-harvest management of aflatoxin contamination in groundnut in West and Central Africa. In abstracts of a conference on *Reducing Impact of Mycotoxins in Tropical Agriculture with Emphasis on Health and Trade in Africa*. Accra, Ghana. International Institute of Tropical Agriculture and Myco-Globe, pp20-21

Waliyar, F., Reddy, S.V. and Kumar, P.L*. 2005*. Aflatoxin contamination in maize in Andhra Pradesh, India. In Abstracts 2nd Global Conference on Plant Health – Global Wealth, Rajasthan College of Agriculture and Technology, Udaipur, India

Waliyar, F., Reddy, S.V. and Kumar, P.L. 2005. Estimation of *Aspergillus flavus* Infection and Aflatoxin Contamination in Seeds: Laboratory Manual. International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, India. pp26 Methods manual

Waliyar, F., Siambi, M., Jones, R., Reddy, S.V., Chibonga, D., Kumar, P.L., Denloye, S. and **Chinoko, Y.** 2005. Instiutionalization of aflatoxin testing facility in Africa. In abstracts of a conference on *Reducing Impact of Mycotoxins in Tropical Agriculture with Emphasis on Health and Trade in Africa*. Accra, Ghana. International Institute of Tropical Agriculture and Myco-Globe, pp38.

Yadav RS, Hash CT, Bidinger FR, Rizvi SMH, Kumar PS, Sharma A, Skot KP, Vadez V and **Howarth CJ.** 2005. Physiology, genetics and marker-assisted breeding of drought tolerance QTL in pearl millet. Final Program and Abstract Book, InterDrought-II. The 2nd International Conference on Integrated Approaches to Sustain and Improve Plant Production Under Drought Stress, September 24 to 28, 2005, University of Rome "La Sapienza", Rome, Italy. P. 7.36.

Yadav, R.S., Bidinger, F.R., Hash, C.T., Cavan, G.P., Serraj, R., and **Howarth, C.J.** 2001 (published 2005). Improving pearl millet drought tolerance. International Sorghum and Millets Newsletter 42:15-16

2. Scientists in Biotech Theme activities during 2005

Name	Position
Auna, R	Scientist (Breeding)
Chandra, S	Principal Scientist-Biometrics and Head Bioinformatics
Gaur, PM	Principal Scientist, Breeding
Hoisington, D	Global Theme Leader
Hash, CT	Principal Scientist, Breeding
Jayashree, B	Scientist, Bioinformatics
Kashiwagi, J	Associate Principal Scientist (Crop Physiology)
Kiambi, D	Scientist (Molecular Genetics)
Lava Kumar, P	Special Project Scientist
Mallikarjuna, N	Senior Scientist, Cell Biology
Nigam, SN	Principal Scientist, Crop Improvement
Pande, S	Principal Scientist, Pathology
Reddy, BVS	Principal Scientist, Breeding
Sagnard, F	Principal Scientist (CIRAD, ICRISAT)
Santa de Villiers	Scientist (Cell Biology)
Saxena KB	Principal Scientist, Breeding
Sharma, HC	Principal Scientist (Entomology)
Sharma, KK	Principal Scientist (Cell Biology)
Thakur, RP	Principal Scientist (Pathology)
Upadhyaya, HD	Principal Scientist (Genetic Resources)
Vadez, Vincent	Principal Scientist (Crop Physiology)
Varshney, RK	Senior Scientist (Applied Genomcis)
Waliyar, F	Principal Scientist (Pathology) and Adviser to Director General
Weltzein-Rattunde, E	Principal Scientist, ICRISAT-Mali

3. Visiting Scientists/Consultants

Name	Position
Anjaiah, V	Visiting Scientist
Aparna, V	Visiting Scientist
Dhillon, M	Visiting Scientist
Deshpande, S.P.	Consultant
Dwivedi, SL	Visiting Scientist
Folkertsma, R.	Special Project Scientist (Kenya)
Gulia, Surinder K	Consultant)
Madhusudhanana R	Consultant
Nepoloean, T	Special Project Scientist (Jul-Dec)
Peter Vijay	Consultant
Ramu,	Consultant
Satish Kumar, P	Consultant
Senthivel, S	Special Project Scientist
Wynand van Der Walt	Consultant

4. Biotechnology students

Name	Country	Category	Degree	Title of the topic
Abeysinghe Pushpa Damayanthi	Srilanka	Research Fellow		Training in Molecular characterization of *Arachis* germplasm
Afsha Tanveer S	India	Apprentice	MSc	Wide crosses in the genus C*icer*
Americo Uaciquete	Mozambique	Research Fellow		Training in afllatoxin detection and estimation
Anitha S	India	Apprentice		Detection of aflatoxins in blood samples
Arunkumar Bhatt A	India	Research Fellow		PCR-based RAPD analysis for hybrids and parents of Castor
Arvind Kumar M	India	Apprentice		Bioassay of transgenic chickpea to Helicoverpa
Asha Kiran A	India	Apprentice	MSc	Anther culture in chickpea and pigeonpea
Ashwini T	India	Apprentice		Phylogenetic analysis of stress tentative ortholog groups
Basweti Caleb Ntabo	Kenya	Research Fellow		Training in Afllotoxin detection and estimation
Bhushan Rameshrao K	India	Research Scholar	PhD	PhD thesis on Molecular markers for identification of sorghum varieties and hybrids
Chandra Atika	India	Research Fellow		Transformation of pigeonpea
Chandra Kala I	India	Apprentice	BTech	Comparative study of sequence clustering software
Cotter Sheena	Australia	Research Fellow		Evaluation of wild chickpeas to pod borer resistance
Durgaprasad H D	India	Apprentice		Stay green roots
Eguchi Maki	Japan	Research Fellow		Molecular characterization of genetic resources in chickpea, pigeonpea, finger millet
Elizabeth Jane Newbold	USA	Apprentice		Groundnut IPM
Fatema S H	India	Research Scholar	PhD	PhD thesis on Introgression of fungal disease resistance from wild Arachis
Girija Rani	India	Apprentice	MSc	Tissue culture studies on chickpea
Hanspal Manindra Singh	India	Apprentice		Anotation of tentative orthologous sequence from stress libraries
Joseph Pilirani Maruwo	Malawi	Research Fellow		Training in Afllotoxin detection and estimation
Kassahun Bantte Bisetegn	Ethiopia	Research Scholar	PhD	PhD thesis on Drought tolerance in sorghum
Khopade Parikshit M K	India	Apprentice	MSc	Tri-tophic interactions of natural enemies of Helicoverpa and pigeonpea genotypes
Latha T	India	Apprentice		Groundnut drought research
Madhurima Bhatnagar	India	Research Scholar	Hyderabad	PhD thesis work on Development and cahracterization of transgenic groundnut plants for enhanced production of ß-carotene to combat vitamin A malnutrition

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Name	Country	Category	Degree	Title of the topic
Mahender T	India	Research Scholar	PhD	PhD thesis on Genetic and genomic mapping of pearl millet using EST and other markers for abiotic stress tolerance
Mazibur Rahman AKM	Bangladesh	Research Fellow		In vitro regeneration and genetic transformation of Bt gene in chickpea
Mei Yuan	China	Research Fellow		Genetic transformation techniques in groundnut
Moses Wazingwa Munthali	Malawi	Research Fellow		Training in Afllotoxin detection and estimation
Mutegi Charity K	Kenya	Research Fellow		Training in Aflatoxin detection and estimation
Naga Padmini P	India	Research Scholar	MTech	MTech thesis on Tissue culture and transformation of chickpea for insect resistance
Nahed Hassan Alsakhny	Syria	Research Fellow		Tissue culture and transformation of chickpea
Namita Srivastava	India	Research Scholar	PhD	PhD thesis on Molecular and physiological characterization of genetic variation for salinity tolerance in the core germplasm of pigeonpea and groundnut
Narasimha Reddy P	India	Apprentice		Endophytic effect of *Beauveria bassiana* in sorghum against stemborer
Nita L	India	Apprentice		Molecular and physiological characterizations of drought tolerance
Okeno James A	Kenya	Research Fellow		Evaluation of sorghums for midge resistance
Padmaja T	India	Research Scholar	PhD	PhD thesis on Evaluation of Bt toxins and its metabolites against Helicoverpa
Prashanthi M	India	Apprentice	MSc	Characterization of transgenic chickpea for drought tolerance
Rajalakshmy P	India	Apprentice		Identification of protein motifs in clustered stress transcripts
Ramesh Naidu D	India	Apprentice		Wide crosses in Cicer
Ramu P	India	Research Scholar	PhD	PhD thesis on Development and application of EST-SSR marker in sorghum
Ravi V	India	Apprentice		Root contribution to drought avoidance
Sandeep Reddy N	India	Apprentice	BTech	Analysis of multiple sequence alignment algorithms
Siraj MoqamunDin	Afghanistan	Research Fellow		Training in Aflotoxin detection and estimation using ELISA
Sowmini S	India	Research Scholar	MTech	MTech thesis on Development of transgenics for resistance to Aspergillus flavus in the Lipoxiginase genes
Spandana Murthy T	India	Apprentice		Role of ABA response to drought tolerance in pearl millet

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Name	Country	Category	Degree	Title of the topic
Srilekha C	India	Apprentice	BTech	Comparative study of sequence clustering software
Sunita Choudhary	India	Apprentice		Salinity
Suprabhath Reddy G	India	Apprentice	BTech	Analysis of multiple sequence alignment algorithms
Swathi Reddy K	India	Apprentice		Pysiological and biochemical basis of drought tolerance
Veena Lalasa D	India	Apprentice		Transformation of sorghum for insect resistance
Venkata Sasanka P	India	Apprentice		Screening of interspecific derivatives of pigeonpea for Helicoverpa armigera
Vijaya Lakshmi K	India	Apprentice		Computational characterization of clustered simple sequence repeats from plant ESTs
Vipul N	India	Apprentice		Wide crosses in the genus Cicer
Zaman Shazmira	India	Apprentice		Analysis of chickpea transgenics for insect resistance

5. Successful Research Funding Proposals

Donor	Project Title	Project Coordinator	Grant budget inUS $	Duration
USAID/ABSP II Sathguru/Danforth)	Development of tobacco streak virus resistant sunflower and groundnut	Kiran Sharma	1 Apr 2005 to 30 Sep 2006	34,650
USAID/ABSP II (Sathguru/Danforth)	Pod borer resistant chickpea for Bangladesh	Kiran Sharma	Jun 2005-Dec 2006	25,000
USAID program on Biotechnology and Biodiversity Interface (BBI), The Program for Biosafety Systems (PBS), Western Michigan Univ, USA	Environmental risk management of genetically engineered sorghums in Mali	F Sagnard	1 Oct 2004 to 30 Sep 2007	484,000
Africa Harvest Biotech Foundation Intl. (Gates Foundation)	African Biofortified Sorghum	Dr Mary A Mgonja	1 Oct 2005 to 31 Dec 2009	978,000
Approved in Principle				
BMZ/GTZ	Sorghum/Pearl Millet	Dr Bettina Haussmann	2006-2009	1,355,000
Gates Foundation	CRS proposal with ICRISAT as partner	Eva Rattunde		

6. Submitted Proposals

Project title	Date submitted	Total Budget	Total Budget (in US$ ' 000)	No. of years	Donor	Coordinator
Molecular characterization of genetic resources in chickpea, pigeonpea and finger millet	18-Feb-05	No budget indicated			Japan/CGIAR Scholarship	
Virulence Characterization of the Pearl Millet Downy Mildew Pathogen Isolates - Training in Inoculum Multiplication, Artificial Inoculation, Disease Identification, and Data Recording and Data Analysis to Classify the Isolates into Different Virulence Groups	18-Feb-05	No budget indicated			Japan/CGIAR Scholarship	
Do Roots Contribute to Drought Tolerance of Pearl Millet?	18-Feb-05	No budget indicated			Japan/CGIAR Scholarship	
Effect of Helicoverpa-resistant Bt cotton on performance and fitness of biocontrol agents in India	8-Mar-05	Euro 35100	43		DAAD Scholarship	Dr HC Sharma
Sorghum breeding against several diseases and pests using molecular markers	8-Mar-05				National Natural Science Foundation of China	Dr HC Sharma
Evaluation, validation and introgression of transgenic chickpea and pigeonpea for resistance to the legume pod borer "Helicoverpa armigera"	13-Apr-05	Rs 67.21 lacs	155	3	DBT, GOI	Dr KK Sharma
Development of transgenic groundnut lines with reduced aflatoxin contamination	30-Apr-05	USD 105000	105	3	Eiselen Foundation	Dr KK Sharma
Characterization of Viruses Associated with Yellowing Disease of Chickpea: A Step Towards Attaining Sustainability of Chickpea Production in Iran	30-Jun-05	USD 132, 270	132	3	AERO, Iran	Dr F Waliyar

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Project title	Date submitted	Total Budget	Total Budget (in US$ ' 000)	No. of years	Donor	Coordinator
Exploitation of Wild Relatives of Pigeonpea for Resistance to Pod Borer, Helicoverpa Armigera	24-Jun-05	Rs 57,01,000	130	3	APNLBP	Dr HC Sharma
Characterization and evaluation of transgenic pigeonpea for disease resistance against fungal pathogens-Phase II	19-Jul-05	Rs.42,36,675	97	3	APNL0P	Dr K K Sharma
Confronting the pest with the host: Exploitation of insect-resistant varieties for the management of Helicoverpa in chickpea		US$ 77,400	77	1	DBT, GOI	Dr H C Sharma
Losses due to Stem Borer Damage in Rabi Sorghum and the Role of Bio-Control Agents in Reducing the Need for Pesticide Application	29-Dec-05	US$ 12192.4	12		NRCS	Dr H C Sharma

About ICRISAT®

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a nonprofit, non-political organization that does innovative agricultural research and capacity building for sustainable development with a wide array of partners across the globe. ICRISAT's mission is to help empower 600 million poor people to overcome hunger, poverty and a degraded environment in the dry tropics through better agriculture. ICRISAT belongs to the Alliance of Future Harvest Centers of the Consultative Group on International Agricultural Research (CGIAR).

Contact Information

ICRISAT-Patancheru
(Headquarters)
Patancheru 502 324
Andhra Pradesh, India
Tel +91 40 30713071
Fax +91 40 30713074
icrisat@cgiar.org

Liaison Office
CG Centers Block
NASC Complex
Dev Prakash Shastri Marg
New Delhi 110 012, India
Tel +91 11 32472306 to 08
Fax +91 11 25841294

ICRISAT-Nairobi
(Regional hub ESA)
PO Box 39063, Nairobi, Kenya
Tel +254 20 7224550
Fax +254 20 7224001
icrisat-nairobi@cgiar.org

ICRISAT-Niamey
(Regional hub WCA)
BP 12404
Niamey, Niger (Via Paris)
Tel +227 722529, 722725
Fax +227 734329
icrisatsc@cgiar.org

ICRISAT-Bamako
BP 320
Bamako, Mali
Tel +223 2223375
Fax +223 2228683
icrisat-w-mali@cgiar.org

ICRISAT-Bulawayo
Matopos Research Station
PO Box 776,
Bulawayo, Zimbabwe
Tel +263 83 8311 to 15
Fax +263 83 8253/8307
icrisatzw@cgiar.org

ICRISAT-Lilongwe
Chitedze Agricultural Research Station
PO Box 1096
Lilongwe, Malawi
Tel +265 1 707297/071/067/057
Fax +265 1 707298
icrisat-malawi@cgiar.org

ICRISAT-Maputo
c/o IIAM, Av. das FPLM No 2698
Caixa Postal 1906
Maputo, Mozambique
Tel +258 21 461657
Fax +258 21 461581
icrisatmoz@panintra.com

Visit us at www.icrisat.org

274–2006