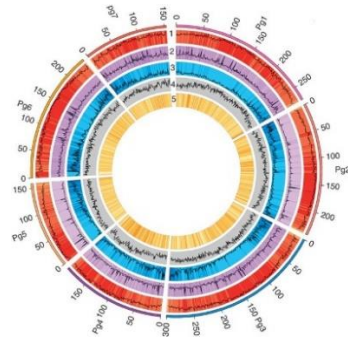


Genetic Gains

Genome decoding

Pearl millet genome decoded and sequenced



The decoding and sequencing of the pearl millet genome by a global team of 65 scientists from 30 research institutions has revealed critical coping strategies. The team reported the ~1.79 Gb draft whole genome sequence of reference genotype Tift 23D₂B₁-P1-P5, which contains an estimated 38,579 genes. The analysis of genetic variability among a thousand pearl millet lines has led to a better understanding of the ability of this dryland cereal to survive soaring temperatures (over 42°C) and its exceptional drought tolerance. The research highlighted the substantial enrichment for wax biosynthesis genes, which may contribute to heat and drought tolerance in this crop. This discovery published in Nature

Biotechnology (<http://dx.doi.org/10.1038/nbt.3943>), on 18 September 2017, may also help develop climate adaptation strategies in other important cereal crops.

This research was co-led by ICRISAT, India; BGI-Shenzhen, China; and the French National Research Institute for Sustainable Development (IRD). The latest innovations in DNA sequencing and analysis were used to identify new genetic tools like molecular markers related to drought and heat tolerance, as well as other important traits (better nutrition profile and pest resistance). This will catalyze breeding efforts to improve this crucial staple food for millions of people in arid and semi-arid Africa and Asia in particular.

292 pigeonpea genomes resequenced



ICRISAT scientists inspect pigeonpea accessions for agronomically important traits and disease resistance. Photo: S Sharma, ICRISAT

In another significant breakthrough, a global team of 19 scientists from 9 institutes re-sequenced the genome or DNA of 292 pigeonpea varieties and discovered new traits such as resistance to various diseases that affect the crop and its insensitivity to photo-period (the duration of daylight hours required for reaching maturity). The research also traces the likely origin of the domesticated pigeonpea

to Madhya Pradesh in Central India. These discoveries were published in the prestigious journal [Nature Genetics](#) (doi:10.1038/ng.3872).

This breakthrough will lead to the development of superior varieties of this important pulse crop which can enable higher production and profits for smallholder farmers, better market value and bring it within an affordable price range for the consumer, especially the poor. Pigeonpea is a major source of protein, fiber, minerals, vitamins and resistant starch. It is important for ensuring nutritional security for millions in the developing world. However, the crop yield has remained stagnant over the last six decades, with limited genetic diversity and genome information, which is required for the development of superior varieties.

The study used re-sequencing data to identify genomic regions impacted by domestication and breeding that have contributed to narrowing the genetic base. The study also identified the genetic origin of the crop at the DNA level for the first time and identified genes with agronomically useful traits such as resistance to Sterility mosaic disease and Fusarium wilt, and insensitivity to photoperiod that will help to accelerate pigeonpea breeding and reduce the time to develop superior varieties from 8-10 years to 5 years.

For more see: <http://www.icrisat.org/genome-re-sequencing-to-accelerate-genetic-gains-of-pigeonpea/>

Downy mildew pathogen genome sequenced

Pearl millet downy mildew pathogen genome was also sequenced ([Biotechnology Reports](#), 2017), and elicitation of resistance in the downy mildew pathogen was reported (Scientific Reports 2017; 7:43991).

Making marker genotyping cost effective

- High-density SNP genotyping arrays with >58K SNPs were developed for chickpea, groundnut and pigeonpea.
- Also filed a provisional Indian Patent (application no.: 201741014402) on 24 April 2017, on molecular markers for the determination of fertility restorer lines in pigeonpea.

Application of genomics in crop breeding



India's first-ever oleic-rich peanuts in Spanish and Virginia bunch types, adapted to Indian farm conditions.

- **16 high oleic lines of groundnut developed** using genomics approach have been nominated to the All India Coordinated Research Project on Groundnut (AICRP-G) and completed the first year

of trial. Three promising molecular breeding lines for foliar disease resistance were promoted from special trial on Near Isogenic Line (NIL) to second and final year advanced varietal trial (AVT-II) of the AICRP-G.

- **HHB 67 Improved** (the first marker-assisted breeding product in the country) continues to grow in more than 800,000 ha (~10% of the pearl millet growing area) in India through the breeder seed production support for the seed (A-/B- lines) and pollen (R- line) parents.
- **10 lines with enhanced drought tolerance**, developed through molecular breeding, were tested after promoting from the advanced varietal trials AVT-I to AVT-II in the All India Coordinated Research Project on Chickpea of the Indian Council of Agricultural Research (ICAR). Three molecular breeding lines that performed better in multi-location trials are being nominated to the state varietal release committee for release as Super Annigeri-1 in Karnataka state.
- **Pigeonpea pre-breeding line, ICPL 15028, having high-yield and must-have traits (resistance to sterility mosaic diseases, and wilt) included in IVT (Initial Varietal Trials) of AICRP** (All India Coordinated Research Project) on Pigeonpea and is used as a donor in the national crossing program of India; besides this, several high-yielding pre-breeding lines were identified in different maturity duration groups and are in various stages of testing for varietal release.

[A novel open reading frame \(orf\) identified to modulate cytoplasmic male sterility in pigeonpea](#)

- The molecular basis of cytoplasmic male sterility in pigeonpea has been deciphered, further strengthening the pigeonpea hybrid breeding technology in this important pulse crop. This provides an answer to the causal gene of pigeonpea cytoplasmic male sterility, which is very crucial for identifying the genes responsible for fertility restoration and purity assessment.
- This research output not only deals with critical knowledge with the A4-based CMS system in pigeonpea but also implicates the identified gene (cDNA) to cause male sterility in a range of crops when expressed in a floral-specific manner. There is a strong incentive to develop such systems that facilitate hybrid production, particularly in crops where hybrid production has been impossible or difficult to develop. A Patent Cooperation Treaty (PCT) has been filed on this disclosure on “Cytoplasmic Male Sterile Gene *ORF147* of pigeonpea and uses thereof” in the field of plant biology and plant breeding.

[Biotechnology for aflatoxin resistance in groundnut](#)

An innovative research successfully demonstrated tangible proof of concept to incorporate high aflatoxin resistance within groundnuts prone to *Aspergillus* infection and subsequent aflatoxin contamination. This collaborative effort involved ICRISAT, Donald Danforth Plant Science Center, Louisiana State University (LSU), and the US Department of Agriculture (USDA). The outputs of this research have shown potential for taming this fungal threat by developing groundnuts free from aflatoxins with a biotechnological double defense strategy ([Sharma et al., 2017](#)).

While one strategy involved production of antimicrobial peptides called defensins using a genetic engineering approach that resulted in no or minimal growth and infections by *A. flavus*; the other strategy, namely, plant-induced gene silencing used small siRNAs that interfered with the toxin production pathway and made the fungus incapable of aflatoxin production and accumulation. In this approach, small RNA molecules from the *Aspergillus* fungus that are involved in the aflatoxin

biosynthetic pathway were made to be produced by the peanut seed that inactivated the target genes in the fungus that are responsible for aflatoxin synthesis.

Both approaches showed great heritability when tested for over three seed generations and remarkable levels of aflatoxin resistance. Using a highly sensitive detection tool (High-Performance Liquid Chromatography), researchers could hardly detect any trace of aflatoxin (below 1 ppb) upon fungus inoculation, compared to the control seeds that accumulated over 3,000 ppb within 3 days post infection. These two traits are now being combined in a single variety to ensure double protection is rendered by both technologies, ultimately leading to healthy peanuts that are immune to *A. flavus* infection and subsequent aflatoxin contamination.

Further study of these aflatoxin-free lines will also help better understand the molecular mechanisms involved, which can be transferred to other crops. Development and deployment of such improved germplasm in an effective manner will also be an important prerequisite for sustainable use of biotechnology for crop improvement and food safety. This will prevent thousands of tons of fungi-affected crops from being discarded or consumed with unacceptable levels of the toxin.

Biotechnology breakthrough can make groundnut immune to aflatoxin

Global burden of aflatoxin

- Upto 25% of liver cancer cases due to aflatoxin
- 5 billion people at risk worldwide
- Stunting in children
- Immune system dysfunction

The fungus *Aspergillus* spp. infects groundnut crop in farmer's field...

Through cracks in pods due to heat/drought/insects

...and throughout the value chain

Groundnut infected with *Aspergillus*

- Livestock feed → Aflatoxin-contaminated meat & milk (India: Aflatoxin in 38% of milk samples over safety limit)
- Processed products → Aflatoxin-contaminated peanut butter (Zambia: 50-80% of groundnut unsafe for human consumption)
- Rejected by export markets (<2-20 ppb aflatoxin limit) → Loss in trade (up to US\$932 million)

A double-defence approach for aflatoxin mitigation

Approach 1: Defensin line

Gene for resisting *Aspergillus* growth taken from alfalfa plant → Introduced into groundnut DNA → 'Defensin' proteins produced → Fungal growth inhibited

Approach 2: HIGS (Host-Induced Gene Silencing) line

Small *Aspergillus* RNA segments introduced in groundnut plant → Fungus attacks groundnut → Aflatoxin-producing gene 'silenced' in *Aspergillus* → Aflatoxin production stopped

Aflatoxin-immune groundnut (<1 ppb 3 days after fungus inoculation) compared to heavily contaminated seeds (over 3000 ppb)

>98.5-99.99% reduction in aflatoxin production

What's next?

Defensin line
×
HIGS line

Field trials and varietal development

Less aflatoxin – better nutrition

Greater market opportunities for farmers

INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS

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