Advances in Food Legumes Research at ICRISAT

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Abstract

The mandate grain legumes of ICRISAT include chickpea, pigeonpea and groundnut which are important crops of Asia and Africa. The grain legumes improvement program of ICRISAT has access to the largest collection of germplasm of these crops (20,602 accessions of chickpea, 13,771 accessions of pigeonpea, and 15,446 accessions of groundnut) available in ICRISAT genebank, state-of-the art genomics lab, Platform for Translational Research on Transgenic Crops (PTTC), precision phenotyping facilities for abiotic and biotic stresses, controlled environment facilities and a global network of research partners. The major objectives of grain legumes improvement include high yield, early maturity, resistance/tolerance to key abiotic and biotic stresses, and market preferred grain traits (size, shape and color). The crop-specific breeding objectives include suitability to machine harvesting and herbicide tolerance in chickpea, development of hybrids in pigeonpea, and enhanced oil yield and quality (high oleic content) and tolerance to aflatoxin contamination in groundnut. The crop breeding programs have been making extensive use of the germplasm, including wild species. The advances in genomics include availability of draft genome sequences, large number of molecular markers, high density genetic maps, transcriptomic resources, physical maps and molecular markers linked to genes/quantitative trait loci for key traits. There are successful examples of introgression of traits through marker-assisted backcrossing in chickpea and groundnut. Transgenics events are available for pod borer resistance in chickpea and pigeonpea and drought tolerance in groundnut. Advances have also been made in use of secondary metabolites for promotion of plant growth, control of insect pests and plant pathogens, and biofortification. The breeding materials and germplasm supplied by ICRISAT have led to release of 160 varieties of chickpea in 26 countries, 91 varieties/hybrids of pigeonpea in 19 countries and 190 varieties of groundnut in 38 countries. Many of these varieties have been adopted widely by farmers and benefitted them in sustainably improving their livilihoods.

Keywords: Breeding, chickpea, groundnut, pigeonpea, pulses

Introduction

The mandate food legume crops of ICRISAT include chickpea (*Cicer arietinum* L.), pigeonpea (*Cajanus cajan* L.) and groundnut (*Arachis hypogea* L.) which are globally grown on over 47 million ha. Over 94% of the area of these crops is in Asia and Africa. These legumes are important sources of protein and calories for millions of people in several of Asian and African countries. These legumes are an integral part of cropping system in the semi-arid tropics mainly because of their ability to produce something of economic value (food or fodder) under extreme conditions and soil ameliorative properties.

The partnership of ICRISAT with the National Agricultural Research Systems (NARS), Advanced Research Institutes (ARIs) and other Research and Development organizations globally has contributed significantly to research and development of grain legumes. This article provides an overview of the research progress made on improvement of grain legumes during recent years.

Genetic resources

Plant genetic resources are the key to the success of crop improvement programs. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India has global responsibility to collect, assemble, conserve, characterize, evaluate, distribute and document the wealth of chickpea, pigeonpea and groundnut genetic variation, for use in crop improvement programs. Therefore, ICRISAT genebank has the largest collection of chickpea (20,602 accessions from 59 countries), pigeonpea (13,771 accessions from 74 countries) and groundnut (15,446 accessions from 92 countries) germplasm. Accessions are conserved as active collection at 4^{o}C and 30% RH to maintain the seed viability above 85% for 15-20 years and base collection at -20^{o}C for about 50 years. ICRISAT also established regional genebanks in Nairobi, Kenya, Niamey, Niger and Bulawayo, Zimbabwe, to conserve germplasm of regional importance and core and mini core collections, reference sets, etc., to meet the research needs of NARS in Africa. As a safety backup, 90% accessions were duplicated at Svalbard Global Seed Vault, Norway.

Germplasm collections were characterized for various morpho-agronomic traits. Wide variation was observed for almost all traits. To enhance use of germplasm in crop improvement representative core (10% of entire collection) and mini core (10% of core or 1% of entire collection) (Upadhyaya *et al*., 2001, 2002, 2003, 2006, Upadhyaya and Ortiz 2001) were formed. The mini core collections are now International Public Goods (IPGs) and 131 sets have been shared with NARS partners in 25 countries. Mini core collections have been used to identify multiple trait-specific, genetically diverse and

agronomically desirable germplasm lines in chickpea, groundnut and pigeonpea (Upadhyaya *et al*., 2013; 2014). Composite collections (1000-3000 accessions) were formed and genotyped with 20-50 SSR markers and genotype based reference sets have been developed in chickpea, pigeonpea and groundnut (Upadhyaya *et al*., 2008 a, b, c).

Crop wild relatives harbor genes for adaptive, agronomic and nutritional traits and resistance to pest and diseases. Using a synthetic amphiploid TxAG6, involving three diploid species of *Arachis*, high yielding cryptic introgression lines with exceptionally high 100 seed weight (up to 130 g) spanish types have been developed (Upadhyaya, 2008). Crosses involving *Cajanus cajan* × *C. scarabaeoides* resulted into a line, ICPL 87162, with high seed protein (up to 32%) compared to control, C 11 (23%) (Reddy *et al*., 1997). Initial characterization of cultivated (8000 accessions at ICRISAT and 6300 in China) and wild *Arachis* (304 accessions of 41 species) germplasm revealed abundant variation in oil content. Using high oil lines identified from mini core collection (Upadhyaya *et al*., 2002), lines with exceptionally high oil content (> 60%) have been identified (Upadhyaya, 2016). Further research on systematic characterization of wild relatives for seed nutritional traits is in progress to identify nutritionally dense types for use in breeding new cultivars.

Some elite germplasm lines were released as cultivars - 22 chickpea, 11 pigeonpea and 17 groundnut accessions were released for commericial production in a number of countries. A chickpea landrace, ICC 11879, was released as a variety in eight Mediterranean countries and ICC 13816 was released in seven countries. ICG 12991, a groundnut accession was released in Mozambique, Malawi, Uganda and Zambia. A vegetable pigeonpea landrace from India (ICP 7035) was released as a cultivar in India, Fiji, Nepal, China and Philippines. Wilt resistant pigeonpea landrace, ICP 8863, was released as *Maruti* in India with a benefit of US$ 75 million in 1996 with 73% internal rate of return (Bantilan and Joshi 1996).

Seeds of germplasm accessions are available free of cost at ICRISAT genebank under Standard Material Transfer Agreement (SMTA) of International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), for research and training purpose. To date, ICRISAT genebank provided 151, 972 samples of chickpea to the researchers in 88 countries, 74, 830 samples of pigeonpea to 113 countries, and 101, 109 samples of groundnut to 96 countries. A total of 375, 217 samples were provided to the researchers within ICRISAT.

Pre-breeding

Like other major crops such as rice and wheat, grain legumes have narrow

genetic base which hinders the genetic improvement of these crops. In contrast, wild species are the reservoirs of many important genes and can be utilized for the genetic improvement of grain legumes. Although high levels of genetic variability for important traits such as morpho-agronomic and nutrition-related traits and high levels of resistance/tolerance to biotic/abiotic stresses is available in wild species, these are not being utilized adequately in breeding programs. The major limitation is due to the linkage drag, differences in ploidy levels, and different incompatibility barriers between cultivated and wild species. Under such situations, pre-breeding provides a unique opportunity to expand primary genepool by exploiting genetic variability present in wild species and cultivated germplasm, and will ensure continuous supply of new and useful genetic variability into the breeding pipelines to develop new cultivars having high levels of resistance and a broad genetic base.

Pre-breeding involves identification of desirable traits and/or genes from unadapted germplasm (exotic landraces and wild species) that cannot be used directly in breeding populations, and to transfer these traits into well-adapted genetic backgrounds resulting in the development of an intermediate set of material which can be used readily by the plant breeders in specific breeding programs. Thus, pre-breeding offers a unique tool to bridge the gap between the germplasm conserved in genebanks and utilized in crop improvement programs (Sharma *et al*., 2013).

Chickpea

Precise evaluation of wild *Cicer* species had identified accessions having high levels of resistance against ascochyta blight (AB), botrytis grey mould (BGM) and dry root rot (DRR). These accessions are being utilized to transfer resistant genes for AB, BGM and DRR into popular chickpea cultivars following interspecific hybridization. The major focus of these activities is to combine heat tolerance with DRR resistance and short duration with BGM resistance. To meet these objectives, the development of AB-QTL populations is in progress. Using complex 3-way crosses [C. arietinum × (*C. reticulatum* × *C. echinosperumum*)], advanced backcross populations have been developed in desi and kabuli chickpea genetic backgrounds (Sharma *et al*., 2016). Considerable variability for morpho-agronomic traits has been observed in these populations (Sharma *et al*., 2016). These populations will provide new and diverse variability for important traits for further use in chickpea improvement programs.

Pigeonpea

Pre-breeding activities are in progress by utilizing the wild *Cajanus* species from secondary and tertiary genepools for pigeonepa improvement. One major achievement of using wild *Cajanus* species is the development of cytoplasmic nuclear male sterility systems (CMS). These CMS systems

have been developed with cytoplasm derived from different wild *Cajanus* species (Saxena *et al*., 2010). Besides this, pre-breeding activities involving wild *Cajanus* accessions as donors and popular pigeonpea varieties as recipients are in progress to develop new genepools for resistance/tolerance to important biotic/abiotic stresses as well as for agronomic and nutrition-related traits. The focus is on the development of advanced backcross populations to minimize the linkage drag associated with utilizing wild species in crop improvement programs. Using secondary genepool species -*C. cajanifolius* (ICPW 29), *C. acutifolius* (ICPW 12 and ICPW 004), *C. scarabaeoides* (ICPW 281); and tertiary genepool species, *C. platycarpus* (ICPW 68) having useful traits such as tolerance to salinity and pod borer resistance (Srivastava *et al*., 2006; Sujana *et al*., 2008; Jadhav *et al*., 2012); and two pigeonpea cultivars (ICPL 87119 and ICPL 85010) advanced backcross populations have been generated at ICRISAT, Patancheru, India (Sharma and Upadhyaya, 2015). The evaluation of these populations for agronomic traits revealed considerable variability for days to flowering, growth habit, pod and seed traits. Promising introgression lines (ILs) having high number of pods per plant, seeds per pod, and 100 seed weight (>20.0 g) have been identified from the population derived from ICPL 85010 x ICPW 004 cross. High yielding ILs derived from *Cajanus acutifolius* and *C. cajanifolius* have been shared with NARS for multilocation evaluation. Preliminary evaluation of different populations has resulted in the identification of ILs having combined resistance against sterility mosaic disease and wilt, and ILs having moderate resistance against phytophthorablight (Sharma and Upadhyaya, 2015). Recently, efforts were initiated to introgress pod borer resistance from two wild species, *Cajanus acutifolius*, and *C. scarabaeoides* into two pigeonpea cultivars, ICPL 87119 and ICP 8863, following simple and complex crosses (Sharma and Upadhyaya, 2015).

Groundnut

Enormous genetic variability is present in genus *Arachis* comprising 80 wild species and cultivated groundnut. Wild *Arachis* species offer significant variability particularly for biotic and abiotic stresses that can be utilized to develop cultivars having enhanced levels of resistance to key stresses and broaden the existing narrow genetic base of cultivated groundnut. The utilization of wild *Arachis* species following interspecific hybridization has resulted in the development of many elite germplasm lines and cultivars with improved level of resistance to diseases and insect-pests. At ICRISAT, several elite lines have been developed with desirable characters transferred from wild *Arachis* species, such as ICGV 86699 (Reddy *et al*., 1996) with resistance to multiple pests, ICGV 87165 (Moss *et al*., 1998) with multiple disease and insect resistance; ICGV 99001 and 99004 with resistance to late leaf spot

(LLS); and ICGV 99003 and 99005 to rust. Besides this, varieties such as ICGV-SM 85048 (Nigam *et al*., 1998), and ICGV-SM86715 (Moss *et al*., 1998), having genetic base from wild *Arachis* species, were released for cultivation, mostly in USA.

Recently, for efficient utilization of diploid wild species from section *Arachis*, several synthetics (amphidiploids and autotetraploids) have been developed by using various A- and B-genome species. These synthetics are being utilized in crossing programs with cultigens to develop pre-breeding population/introgression lines (ILs) having high frequency of useful genes/alleles and good agronomic background.

Evaluation of two such populations derived from ICGV 91114 x ISATGR 1212 (a synthetic derived from *A. duranensis* × *A. ipaensis*) and ICGV 87846 x ISATGR 265 (*A. kempf-mercadoi* × *A. hoehnei*) has led to the identification of ILs having high levels of late leaf spot (LLS) and rust resistance and sufficient genetic variability for morpho-agronomic traits.

These ILs are being genotyped using linked-markers for LLS and rust resistance to identify novel alleles from different wild species other than the commonly used *A. cardenasii* for further use in peanut improvement programs.

Genomic Resources

Chickpea

For faster genetic gains, the availability of genomic resources and their deployment in breeding is a prerequisite. Towards this direction, during the last decade, ICRISAT, in collaboration with several partners at national and international levels, developed several thousands of molecular markers (Nayak *et al*., 2010), high density genetic maps (Thudi *et al*., 2011; Kale *et al*., 2015), transcriptomic resources (Varshney *et al*., 2009; Hiremath *et al*., 2012; Kudapa *et al*., 2014) and physical map (Varshney *et al*., 2014a). Both linkage and linkage disequilibrium mapping based approaches were adopted for understanding the genetics of drought and heat tolerance. As a result, a "*QTL-hotspot*" harbouring quantitative trait loci (QTLs) for several drought tolerance related traits (Varshney *et al*., 2014b), and more than 300 significant marker-trait associations for drought and heat have been identified. In addition, QTLs for key production constraints like fusarium wilt, ascochyta blight (Sabbavarapu *et al*., 2013) and salinity (Pushpavalli *et al*., 2015a) have been mapped. In addition, several functional genomics approaches such as RNA-seq, Massive Analysis of cDNA Ends (MACE) with parental genotypes of mapping populations as well NILs have provided some candidate genes for drought tolerance that are being

validated through genetical genomics and/or TILLING approaches.

For deploying SNP markers in chickpea breeding programs, cost effective SNP genotyping assays like VeraCode and KASPar assays (Roorkiwal *et al*., 2013) were developed. In addition to unravelling the draft genome sequence of chickpea (Varshney *et al*., 2013a), several germplasm lines have been re-sequenced, for instance, parental genotypes of chickpea mapping populations (Thudi *et al*., 2016), 129 released varieties (unpublished), chickpea reference set (unpublished) and multi-parent advanced generation intercross (MAGIC) population (unpublished). Very recently, ICRISAT, along with its partners, has launched the large scale re-sequencing initiative "The 3000 Chickpea Genome Sequencing Initiative" to re-sequence 3000 lines from chickpea composite collection. This initiative enabled identification of several million SNPs, Indels, copy number variations (CNVs), and presence absence variations (PAVs) that can be deployed in chickpea improvement programs. A precise and cost-effective SNP genotyping platform with 50,590 high quality non-redundant SNPs on Affymetrix® Axiom® genotyping array was developed. This array will be useful for fingerprinting the released varieties as well as assessing their adoption in addition to genetics and breeding applications. The genomic resources have been successfully deployed for developing superior lines with enhanced drought tolerance (Varshney *et al*., 2013b), and fusrium wilt and ascochyta blight resistance (Varshney *et al*., 2014c). This success story has led to the introgression of the "*QTL-hotspot*" region into several elite varieties in India as well as Kenya and Ethiopia. Further, the available genomic resources also enabled the successful deployment of modern breeding approaches like genomics selection for faster genetic gains.

Pigeonpea

To exploit full potential of genomics for pigeonpea improvement, a significant amount of genomic resources have been developed. For instance, a draft of the nuclear and the complete mitochondrial genome sequence (Varshney *et al*., 2012; Tuteja *et al*., 2013), large repertoire of molecular markers (Saxena *et al*., 2014), high throughput genotyping platforms, transcriptome assembly (Kudapa *et al*., 2014) and genetic maps (Bohra *et al*., 2012) have been developed. The draft genome sequence at the very first instance enriched pigeonpea with information on protein coding genes, more than 54,000 Simple Sequence Repeat (SSR) markers and more than 12,000 high quality Single Nucleotide Polymorphisms (SNPs). Further, the availability of draft genome sequence has allowed implementing advanced methodologies such as whole genome re-sequencing (WGRS), genotyping by sequencing (GBS) and high density SNP array in pigeonpea. A number of WGRS projects initiated and

sequencing data have been generated for more than 400 pigeonpea lines representing the reference set (unpublished), parental lines of hybrids (unpublished) and parental lines mapping populations segregating for economical important traits (Kumar *et al*., 2016). The detailed analyses of WGRS data in three different sets have provided long awaited genome-wide variations to overcome the low level of marker polymorphism in pigeonpea. In summary, WGRS data analysis provided unique accession signatures, targets of domestication and human selection associated genetic sweeps, information on centre of origin and marker trait associations (MTAs) for days to 50% flowering, days to 75% maturity, number of seeds per pod, 100 seed weight, etc. Apart from these, GBS has been deployed to generate high density genetic maps for many inter- and intra-specific populations (unpublished). These high density genetic maps along with multi-year trait phenotyping data will provide MTAs for sterility mosaic diseases, fusarium wilt, fertility restoration, yield related traits, etc. All these genomic resources along with the above mentioned approaches will be used for strengthening the pigeonpea breeding.

Groundnut

Genomics-assisted breeding (GAB) has demonstrated promising results in improving few traits in several crops with high precision leading to the accelerated development of improved lines. However, the availability of an optimal level of genomic resources is must for deploying GAB in any crop species including groundnut. This crop suffered for several years for achieving an optimal level of genomic wealth for conducting genetic and molecular breeding studies. The overview presented below is regarding the development of genomic resources and their deployment in groundnut breeding.

Quick evolution innext-generation sequencing (NGS) technologies have drastically reduced cost of sequencing, encouraging researchers to develop good quality genome assembly for crops with large sized genome such as groundnut. Since cultivated groundnut is tetraploid containing two sub-genomes (A and B), draft genome assembly has been developed for diploid progenitors i.e., *Arachis duranensis* (A subgenome) and *A. ipaensis* (B subgenome). ICRISAT collaborated with International Peanut Genome Initiative (IPGI) for decoding draft genome for both the diploid progenitors, while co-led another initiative -Diploid Progenitor Peanut A-genome Sequencing Consortium (DPPAGSC) for sequencing the A-genome progenitor. The above collaborative effort made available two genome assemblies for A subgenome (*A. duranensis*, accession V14167 and PI475845) and one assembly for B subgenome (*A. ipaensis*, accession K30076) in 2016 (Bertioli *et al*., 2016; Chen *et al*., 2016). The genome size of A

subgenome and B subgenome were found to be 1.1 and 1.38 Gb, respectively. The availability of these assemblies together with re-sequencing data of limited genotypes will provide much needed boost to the several ongoing genetic and breeding studies in groundnut. Such resources also opened possibilities to deploy several modern genomics studies for faster gene discovery and trait improvement in coming years. Till very recently, only ~5,000 simple sequence repeat (SSR) markers were available in public domain (Pandey *et al*., 2012, Varshney *et al*., 2013c). ICRISAT in collaboration with DArT Pty Ltd, Australia, developed diversity arrays technology (DArT) arrays with 15,360 features and Kompetitive Allele Specific PCR (KASP) assays for 90 SNPs in groundnut (Varshney 2015; Janila *et al*., 2016a; Pandey *et al*., 2016). Due to the availability of genome sequence for both the ancestors of cultivated tetraploid, now a large number of SSR markers and millions of single nucleotide polymorphisms (SNPs) have become available for use in genetics and breeding applications. More recently, ICRISAT has developed 58K SNP array using Affymetrix SNP platform covering the entire genome whith a very efficient and high throughput genotyping tool for conducting high resolution trait mapping and modern breeding such as genomic selection (Varshney 2015; Pandey *et al*., 2016).

ICRISAT has been the pioneer in developing genetic maps and conducting QTL analysis for identification of linked markers in groundnut. For example, ICRISAT developed five SSR based genetic maps using recombinant inbred line (RIL) populations, and one DArT/DArTseq based genetic map using F_2 mapping population with 1,152 loci in addition to the first SSR based genetic map (Varshney *et al*., 2013c; Janila *et al*., 2016a; Pandey *et al*., 2016). Moreover, ICRISAT also developed a consensus genetic map for the first time with 897 marker loci which was then improved to 3,693 marker loci. In addition, ICRISAT has also collaborated with USDA-ARS, Tifton and developed two improved genetic maps for two RIL populations (Varshney 2015; Pandey *et al*., 2016). Further, using linkage mapping and genome-wide association studies (GWAS), a large number quantitative trait loci (QTLs)/marker-trait associations (MTAs) were identified for drought tolerance related traits, late leaf spot resistance, rust resistance, oil content, oil quality, yield related traits, physiological traits and seed dormancy (Varshney *et al*., 2013c; Varshney 2015; Janila *et al*., 2016a; Pandey *et al*., 2016). Linked markers for rust resistance, late leaf spot resistance and high oleic acid were validated successfully and deployed in molecular breeding. The first example of molecular breeding at ICRISAT was improvement of three popular varieties, namely ICGV 91114, JL 24 and TAG 24, for rust resistance using the marker-assisted backcrossing (MABC) approach (Varshney *et al*.,

2014d). The field evaluations of these MABC lines recorded increased pod yields (56–96%) and also retained early maturity duration. Of these lines, six best MABC lines, namely ICGV 13192, ICGV 13193, ICGV 13200, ICGV 13206, ICGV 13228 and ICGV 13229, were picked with 39–79% higher mean pod yield and 25–89% higher mean haulm yield in comparison to their respective recurrent parents (Janila *et al*., 2016b). Some of these MABC lines have now been nominated to the special trial on Near Isogenic Line (NIL) of the All India Coordinated Research Project on Groundnut (AICRP-G) for evaluation and release. The second successful example of molecular breeding in groundnut was improvement of three groundnut varieties, namely ICGV 06110, ICGV 06142 and ICGV 06420, for oil quality (high oleic acid, low linoleic acid and low palmitic acid) using two approaches, namely MABC and marker-assisted selection (MAS). Linked gene-based markers were used to introgress two mutant alleles from the SunOleic 95R carrying two FAD2 mutant alleles responsible for oil quality traits. These lines showed elevated oleic acid (62 to 83%), i.e., oleic acid increased by 0.5–1.1 folds along with reduced linoleic acid by 0.4–1.0 folds and palmitic acid by 0.1–0.6 folds (Janila *et al*., 2016c). Several of these lines were selected for further multilocation yield trials in order to select promising lines for nomination to the AICRP-G for further evaluation and release.

Physiology

Much progress has been made over the last decade or so in our understanding of the adaptation of grain legumes to major abiotic stresses such as water deficit (‘drought’) and soil salinity.

Drought

Much of the efforts have been focused on chickpea and groundnut, involving a cross-species comparison between bean and cowpea in some aspects, and only recently has some work been initiated in pigeonpea.

In the case of chickpea, initial work had involved the screening of chickpea for long and profuse rooting system to allow plants to extract more water from the soil profile. This work has started by the identification of a large genetic variation for root traits (Kashiwagi *et al.*, 2005), followed by the development of mapping populations and the identification of a major QTL for root traits on linkage group 4 of chickpea (Varshney *et al*., 2013b). Building up on this work, a lysimeter system has been developed (Vadez *et al*., 2008) allowing to go beyond measuring roots and allowing to measure water extraction from the soil profile. The system has been used in chickpea germplasm contrasting for their “drought tolerance” based on seed yield under terminal stress (independent of flowering time) and this work has shown that tolerant and sensitive material did not extract different amounts of water from the soil profile (Zaman-Allah *et al*., 2011a). Rather, tolerant materials were

able to extract somewhat less water at vegetative stage than sensitive germplasm, and then had more water left for reproduction and grain filling stages. Additional research showed this was possible because of: (i) a canopy that developed slower; (ii) lower canopy conductance at vegetative stage, especially under high vapor pressure deficit (VPD) conditions (Zaman-Allah *et al*., 2011b). This work has been backed up by crop simulation work that has shown indeed that an early water extraction by a more vigorous phenotype could be detrimental in certain situations, but not under short cycle environments like South India (Vadez *et al*., 2012c). The current focus of that work is to identify QTL for the canopy conductance and development characteristic, using a high throughput phenotyping platform developed to that end (LeasyScan – Vadez *et al*., 2015), the basic idea being to fit ideotypes to specific environments on the basis of their water requirements.

In the case of groundnut, much of the work of the last three decades or so has focused on the identification of genotypes with high transpiration efficiency (e.g. Rao *et al*., 1993; Wright *et al*., 1994). This work has relied mostly on the use of surrogate traits for TE, i.e. SPAD chlorophyll meter readings – SCMR, specific leaf area – SLA, or the carbon isotope discrimination-CID. However, a recent evaluation of TE in a large set of groundnut germplasm, using a lysimeter system–therefore a gravimetric assessment of transpiration efficiency (TE) with no surrogate use–led to an important finding: surrogate traits were not related to TE in any way, regardless of water treatment or sampling time (Vadez and Ratnakumar, 2016). The ruling hypothesis in the past two decades was that higher TE would be driven by a higher photosynthetic rate in groundnut and each of these surrogates, SCMR, SLA, or CID, indirectly proxies for differences in the photosynthetic rate. The finding of an absence of a relationship between a gravimetric/robust TE measurement and the surrogates is an indication that high TE is driven by something else.

According to the theory (Condon *et al*., 2002), high TE is driven either by a high photosynthetic rate or by a low stomatal conductance. Genetic variation has been recently found in groundnut for the capacity to restrict transpiration under high VPD (Devi *et al*., 2010). The TE differences identified in the large germplasm assessment are likely explained by differences in the transpiration control under high VPD (see discussion in Vadez and Ratnakumar, 2016). As in the case of chickpea (and other crops), the current research on groundnut adaptation to water deficit therefore focuses on fitting ideotypes to water availability (Halilou *et al*., 2015). Research has also been carried out to identify genetic variation for adaptation to intermittent drought (Hamidou *et al*., 2012).

Crop simulation is also used as an important entry point to characterize the environments with regards to stress intensity. Then research focuses on analyzing genetic variation in traits that contribute to the plant water budget, those involved in the dynamic of canopy development as in chickpea and those involved in the regulation of stomata opening, using the LeasyScan platform to measure these traits in a high throughput manner (Vadez *et al*., 2015).

Soil salinity

Much of the efforts have been focused on chickpea, where initially a large variation for salinity tolerance was identified (Vadez *et al*., 2007), from which donor parents were chosen for breeding and used to better understand salinity tolerance traits. Two major finding helped in this search: (i) the first was an absence of relationship between the seed yield under salt stress and vegetative growth at about flowering time - this finding dismissed the idea that early screening at germination or vegetative stage could be carried out, and also implied salinity tolerance had a close link with the reproductive biology of the plant; (ii) the absence of a relationship between the sodium (Na) accumulation in the shoot tissue at vegetative stage and the degree of tolerance based on seed yield under stress – a finding that dismissed the hypothesis of a Na toxicity. In follow up research, it was found that salt tolerance was related to the capacity of tolerant genotypes to maintain a higher number of fertile pods (Vadez *et al*., 2012a), something that was confirmed later (Pushpavalli *et al*., 2015a).

Among the germplasm that was tested, parents of a mapping population showed contrast under salt stress and screening of the population led to the first QTL for salinity tolerance in chickpea (Vadez *et al*., 2012b). Another population was later used and additional QTLs were identified, with a particular interest on two genomic regions harboring a high number of genes involved in the response to salt stress (Pushpavalli *et al*., 2015a). One pending aspect has been the focus of the last few years of research: the fact that Na had no toxicity effect led us to hypothesize that chloride (Cl) anions could have such a toxic effect.

Research was undertaken to test this hypothesis, testing also ions (Cl, Na, K) level in different plant organs, including the reproductive parts and no relationship was found between tolerance and ion level in any of the plant part (Turner *et al*., 2013; Kotula *et al*., 2015; Pushpavalli *et al*., 2016).

Therefore, there is still quite a bit of "mystery" around the reasons for salinity tolerance in chickpea, although it is now well established that it involves tolerance of the reproductive biology, independently of any ion toxicity, and that large variation in the tolerance exists and genomic regions involved in that tolerance have been identified.

Pathology

Chickpea

The production and productivity of chickpea is severely constrained by diseases such as Fusarium wilt (FW, *Fusarium oxysporum* f sp *ciceris*), dry root rot (DRR, *Rhizoctonia bataticola*), Ascochyta blight (AB, *Ascochyta rabiei*) and Botrytis gray mold (BGM, *Botrytis cinerea*). These diseases have been reported to cause huge losses in susceptible cultivars under favorable environmental conditions (Choudhary *et al*., 2013; Ghosh *et al*., 2013). Advances have been made in the areas of host plant resistance, host x pathogen x environment interactions and pathogenomics to understand the resistance mechanism in these diseases in chickpea. Stepwise screening procedures for these diseases (greenhouse and field) have been reported by Pande *et al*. (2012a).

Recent studies have indicated changes in the race scenario of pathogen and existence of multiple races (Sharma *et al*., 2014). Stable and broad based sources of resistance to wilt (ICCV 05527, ICCV 05528 and ICCV 96818) have been identified through the multi-year and multi-location evaluation (Sharma *et al*., 2012a). Genetics of resistance against different races has been studied in detail, and contradicting results have been reported (compiled by Choudhary *et al*., 2013). Progress has been made in molecular breeding for wilt resistance and tagging of wilt resistant genes through molecular markers (Varshney *et al*., 2014). Dry root rot is found to be an emerging disease in chickpea particularly and is predisposed by high temperature and soil moisture stress (Sharma and Pande, 2013; Sharma *et al*., 2015a). Recent surveys conducted during 2010–2013 in India indicated widespread and increased incidence of DRR in the central and southern states of India (Ghosh *et al*., 2013). Cultural, morphological and molecular variations in 94 isolates of *R. bataticola* collected from various agro-ecological zones of India have been reported by Sharma *et al*. (2012b & c). Lack of resistance in the available germplasm and breeding lines is a biggest challenge in managing this disease. Search for specific resistance to DRR in chickpea is continued and few moderate sources of resistance have been identified (ICCV 08305, ICCV 05530 and ICCV 05529). Efforts are underway to develop improved breeding lines/introgression lines (ILs) with enhanced level of resistance to dry root rot and share these promising lines with NARS for use in chickpea breeding programs

Considerable progress has been made in understanding the AB and BGM diseases in chickpea. Moderate resistance to AB has been found in chickpea and breeding for resistance is making progress by identifying new resistance genes. Molecular markers associated with major QTLs conferring resistance to AB have been located on linkage maps, and these

markers can be used for efficient pyramiding of the traits of interest. Pande *et al.* (2012b) identified five genotypes with consistent resistant reaction to AB (EC 516934, ICCV 04537, ICCV 98818, EC 516850 and EC 516971) in multi-environment. In BGM also, only moderate sources of resistance are available (ICCV 96859, ICCV 96853, ICCV 05604, ICCV 96852 and ICCV 05605) (Sharma *et al*., 2013). *Cicer echinospermum* and *C. reticulatum,* the only two compatible annual wild species, have been reported to have resistance to BGM. Hence, interspecific populations were developed with susceptible cultivars as female parents and *C. echinospermum* accession IG 73074 and *C. reticulatum* accession IG 72937 as the pollen donors to transfer and assess the nature of genetic control for BGM. Screening the progeny indicated that resistance to BGM was controlled by a single additive gene/allele (bgmr1cr and bgmr1ce), which can be introgressed through a backcross breeding programme (Ramgopal *et al*., 2013).

Pigeonpea

Fusarium wilt (FW, *Fusarium udum*) and sterility mosaic disease (SMD) caused by pigeonpea sterility mosaic virus (PPSMV) are the most important diseases of pigeonpea and can cause yield losses up to 100% (Saxena *et al*., 2010). Apart from wilt and mosaic, Phytophthora blight (PB, *Phytophthora cajani*) is another important disease that got the status of economic concern (Sharma *et al*., 2006 and 2015b). FW and SMD incidence differs from place to place due to variability in pathogen. Considerable variability have been observed using 73 isolates and 11 differentials collected from seven states in India (Sharma *et al*., unpublished). Three distinct strains (Bangalore, Patancheru and Coimbatore) have been characterized for PPSMV in India (Kulkarni *et al*., 2003). So far, no confirmed information regarding pathogen variability is available for *Phytophthora cajani*.

Reliable greenhouse and field screening techniques are available for FW and SMD to identify resistance sources (Pande *et al*., 2012c). Recent advances in FW and SMD research have facilitated the selection of high-yielding varieties with durable resistance to FW and SMD. Lines derived from crosses with *C. acutifolius* and *C. platycarpus* have shown resistance to the Patancheru isolate of PPSMV under field conditions (Mallikarjuna *et al*., 2011). Recently new sources of resistance to FW and SMD were identified in a mini-core collection of pigeonpea germplasm (Sharma *et al*., 2012d). In multi-environment field testing, four genotypes (ICPLs 20094, 20106, 20098 and 20115) have been identified as the most stable and resistant to SMD (Sharma *et al*., 2015c). Three genotypes (ICPLs 20096, 20107, 20110) showed moderately stable performance against SMD. All these lines have medium duration of

maturity and could be valuable sources of resistance for a pigeonpea breeding programs to FW and SMD. Recently, Sharma *et al*. (2015b) developed a reliable and repeatable zoospore screening technique for PB screening. Using this zoospore bioassay, over 800 pigeonpea genotypes including released cultivars, earlier reported PB resistant lines, breeding lines and water logging tolerant lines have been screened. Repeated screening of promising genotypes has so far identified four genotypes with a moderate resistance to PB (ICPLs 99004, 99008, 99009 and 99048) (Sharma *et al*., unpublished).

Saxena *et al*. (2012) reported dominant suppressive epistatic effect of a dominant gene over the recessive one for wilt resistance in a cross of a FW susceptible cytoplasmic male-sterility line with four FW resistant fertility restorers. The nature of inheritance of SMD was studied in the segregating population of two crosses, Gullyal white (susceptible) X BSMR 736 (resistant) and BSMR 736 (resistant) X ICP 8863 (susceptible) (Bhairappanavar *et al*., 2014;), indicating that the resistant trait was governed by two independent non-allelic genes, designated *SV1* and *SV2*, with inhibitory gene interaction (Bhairappanavar *et al*., 2014). The limited reports available on genetics of PB resistance in pigeonpea suggest that a few major genes may control resistance in the host to PB.

Groundnut

Foliar fungal and viral diseases

Foliar fungal diseases in groundnut such as leaf spots (early and late) and rust and viral diseases such as groundnut bud necrosis disease (GBND), and groundnut rosette disease (GRD) are economically important yield limiting biotic constraints with worldwide significance. Host plant resistance is a cost-effective and sustainable management option for smallholder farmers in fighting these important diseases. Recently ICRISAT scientists identified several groundnut lines from mini core germplasm accessions such as ICGs 4389, 6993, 11426, 4746, 6022 and 11088 with combined good levels of resistance and yield to rust and late leaf spot (Sudini *et al.*, 2015b). Efforts in breeding resistant varieties to groundnut rosette disease (GRD), an important virus disease in sub-Saharan Africa, were successful and lead to the release of several varieties in Africa. For example, ICGV-SM 90704, ICG 12991, ICGV-SM 99568, ICGV 93437, SAMNUT 23, SAMNUT 21 and SAMNUT 22 (Waliyar *et al.*, 2007b).

Aflatoxin contamination

Aflatoxin contamination in groundnut is the most important qualitative problem affecting its profitability, trade and health of humans and animals. ICRISAT has given top priority since 1990's and made tremendous progress in understanding and mapping the occurrence of

aflatoxin contamination in groundnut value chains in various countries in sub-Saharan Africa (SSA) and Asia, identifying pre-harvest and post-harvest interventions to better manage this menace, cost-effective diagnostics to quantify aflatoxins from agricultural commodities and capacity building of NARS of SSA and Asia.

Aflatoxin diagnostics: ICRISAT scientists devised a simple and affordable testing assay using in-house developed antibodies that helps identify aflatoxin-free grains to meet international market standards and ensure higher returns for farmers, and provide safer products for consumers. The test uses a competitive enzyme-linked immunosorbent assay (cELISA) to detect the presence of aflatoxins. The assay has drastically reduced the cost of testing crops from $25 to $1 per sample and can be used with minimal laboratory facilities (Waliyar *et al.,* 2005; Waliyar *et al.,* 2009). Its advantage is that most of the required chemicals are locally available in developing countries and it allows the analyses of up to 200 samples per day. Further we transferred this technology to several NARS partners in Asia and sub-Saharan Africa and significantly contributed to the capacity building of scientific staff and organizations. For example, the National Smallholder Farmers' Association of Malawi (NASFAM) has successfully used the new technology, in conjunction with HPLC, as part of a broader effort to regain its once-lucrative European groundnut export market. ICRISAT recently developed a low cost (<2 USD) rapid test kit too based on lateral flow immunoassay principle for the estimation of aflatoxins in groundnuts. Adding a mobile sample extraction kit to this device will make it the first on-site testing kit for aflatoxins.

Progress in breeding for resistance to aflatoxin: Researchers at ICRISAT were able to identify resistant sources and combine resistance to pre-harvest seed infection and/or aflatoxin contamination into improved genetic backgrounds (Waliyar *et al.,* 1994; Upadhyaya *et al.,* 2001; Upadhyaya *et al.,* 2003; Nigam *et al.,* 2009). In spite of high genotype by environment interaction, a number of germplasm with high levels of resistance across environments, for example, ICGs 1326, 1859, 3263, 4749, 7633, 9407, 9610, and 10094 (Nigam *et al.,* 2009), have been identified in cultivated groundnut. More importantly, some of the germplasm lines such as ICGs 7, 23, 1323, 2925, 5158, 5195, 6760, 9610, 10094, 10609, 10615, 11480, and 11682 were reported to contain very low aflatoxin (0.4 -1.0 ppb) in comparison 171.4 to 304.6 ppb in susceptible controls (Fleur 11 and JL 24). Further a number of breeding lines showed much less pre-harvest aflatoxin contamination levels (0.2 – 4.1 µg kg^{-1} seed) than susceptible control under ambient conditions. Terminal drought predisposes groundnut to *Aspergillus flavus* infection and aflatoxin contamination (Waliyar *et al.,* 2005). Current

breeding research at ICRISAT is focused on development of breeding lines combining early maturity, tolerance to terminal drought and resistance to seed infection and aflatoxin contamination. Genetic crosses were made to generate appropriate breeding populations to select for tolerance to terminal drought and resistance to seed infection and resistance to aflatoxin contamination into improved early maturity genetic background. A number of breeding lines combining short duration and aflatoxin resistance were identified for further evaluation. Some of these varieties in advanced trials were significantly superior (2.8-4.8 t ha^{-1} pod yield) over the control J 11 (2.1 t ha^{-1} pod yield). ICGV 10038 (4.5 t ha^{-1}, 5 µg kg^{-1} aflatoxin content and 5% *A. flavus* infestation), and ICGV 10043 (4.0 t ha^{-1}, 15 µg kg^{-1} aflatoxin content and 2% *A. flavus* infestation) were the best performing entries for pod yield and aflatoxin contamination. Furthermore, ICGVs 13142, 13125, and 13127 combined short duration and resistance to aflatoxin contamination into improved genetic background (ICRISAT Legumes Archival Report 2012/2013).

Agronomic practices for aflatoxin management: Tremendous progress has been made by ICRISAT scientists in identifying pre- and post-harvest cultural practices and testing on-farm which significantly reduce pre and post-harvest aflatoxin contamination. For example, soil amendments (eg. farmyard manure, lime, and gypsum), moisture conservation techniques, pod drying methods and storage methods (Waliyar *et al.,* 2007a; Sudini *et al.,* 2015a).

Monitoring aflatoxin contamination: Continuous monitoring of aflatoxin contamination in food commodity value chains is essential in safeguarding public health. In this direction, ICRISAT conducts regular surveys and map the risk of aflatoxin contamination in target countries of Asia and sub-Saharan Africa. Our findings inform policy makers about the options to best contain this important food safety menace (Monyo *et al.,* 2012; Waliyar *et al.,* 2015; Njoroge *et al.,* 2016).

Integrated Breeding

Chickpea

The major breeding objectives in chickpea include: (1) Enhanced yield potential, (2) Early to extra-early maturity, (3) Tolerance to terminal drought and heat stresses, (4) Resistance to root (fusarium wilt, dry root rot) and foliar diseases (ascochyta blight and botrytis grey mold) and pod borer (*Helicoverpa armigera*), (5) Improved physical (size, shape and color) and nutritional quality (protein and minerals) of grains, and (6) Suitability to machine harvest and tolerance to herbicides.

The chickpea breeding program has been making extensive use of the genetic resources available in ICRISAT's Genebank. Over 20,000

crosses have been made in chickpea so far utilizing over 4,000 unique germplasm accessions (about 20% of the germplasm accessions available in ICRISAT's genebank). ICRISAT is taking three crop cycles per year for rapid generation turnover and accelerating genetic gain. Selection for simple traits is carried out in early segregating generations and for complex traits in F4 and later generations.

We need to improve the precision and efficiency of breeding programs by integrating novel approaches for enhancing genetic base of the breeding populations, genomics-assisted breeding, precision phenotyping, rapid generation turnover and efficient data management system, such as Breeding Management System (BMS) of integrated Breeding Platform (https://www.integratedbreeding.net/)

Tolerance to abiotic stresses

The major abiotic stresses to chickpea production include terminal drought and temperature extremes (low and high). One of the strategies for managing terminal drought and heat stresses is to develop early maturing varieties that can escape these stresses (Gaur *et al*., 2008c, 2015c). Excellent progress has been made in development of early maturing varieties with high yield potential and resistance to fusarium wilt.

Efforts are being made to develop varieties with improved drought avoidance (dehydration postponement) and/or drought tolerance (dehydration resistance) for improving grain yield under drought stress (Gaur *et al*., 2008b). It was found that partitioning coefficient provides an effective selection criterion for grain yield under terminal drought condition (Krishnamurthy *et al*., 2013). Several studies conducted at ICRISAT demonstrated that a prolific root system contributes positively to grain yield under terminal drought conditions (Kashiwagi *et al*., 2013, 2015). However, it is challenging to breed for improved root traits because the screening for root traits is a destructive and labor intensive and difficult to use in large segregating populations. Marker-assisted breeding is ideal for improving such traits. Remarkable progress has been made in development of molecular markers and expansion of genome map of chickpea in recent years (Gaur *et al*., 2012a, 2014c). A genomic region, called QTL-hotspot, carrying several QTLs that are associated with several drought tolerance related traits including some root-traits was located on LG04 (Varshney *et al*., 2014b). This genomic region has been introgressed in several cultivars (JG 11, ICCV 10, JAKI 9218, JG 6) using marker-assisted backcrossing (MABC) (Varshney *et al*., 2013b, Gaur *et al*., 2013b). Several of these lines are being evaluated in the trials of All India Coordinated Research Project (AICRP) on Chickpea.

Heat stress at reproductive stage is increasingly becoming a serious constraint to chickpea productivity

because of large shift in chickpea area from cooler long-season environments to warmer short-season environments, increasing chickpea area under late sown conditions due to increasing cropping intensity, and high fluctuations in temperatures due to climate change (Gaur *et al*., 2014a,b; 2015b). A simple and effective field screening technique for reproductive stage heat tolerance in chickpea has been developed (Gaur *et al*., 2012b, 2014b). A large genotypic variation for reproductive stage heat tolerance has been observed in chickpea (Krishnamurthy *et al*., 2011a, Gaur *et al*., 2014a,b, 2015a) and several heat tolerant genotypes have been identified (ICC 1205, ICC 1356, ICC 4958, ICC 6279, ICC 15614, ICCV 07104, ICCV 07105, ICCV 07108, ICCV 07109, ICCV 07110, ICCV 07115, ICCV 07117, ICCV 07118, ICCV 98902, JG 16, GG 2, JG 130, JAKI 9218, JGK 2 and KAK 2). A heat tolerant breeding line ICCV 92944 has been released as JG 14 in India, Yezin 6 in Myanmar, Chania Desi 2 in Kenya and BARI Chola 10 in Bangladesh.

Tolerance to biotic stresses

Among diseases, fusarium wilt (FW), dry root rot (DRR), and collar rot (CR), are the important root diseases of chickpea in areas where the growing season is dry and warm, while ascochyta blight (AB), and botrytis grey mold (BGM), are the important foliar diseases in the areas where the growing season is cool and humid. Development of FW resistant cultivars is one of the greatest success stories of chickpea breeding and all breeding lines developed at ICRISAT have high resistance to FW. Dry root rot (DRR) has emerged as a major disease under high temperature ($>30^0$ C) and dry soil conditions (Sharma *et al*., 2015a), but development of varieties with high level of resistance continued to remain a challenge due to lack of high level of resistance available in the germplasm.

AB is a highly devastating foliar disease as its pathogen is highly variable and has capability to change and infect newly introduced resistant cultivar (Pande *et al*., 2005). Methods for AB resistance screening have been standardized (Pande *et al*., 2010). Several cultivars with moderate to high levels of resistance have been developed and some breeding lines from multiple crosses (e.g. ICCV 04512, ICCV 04514 and ICCV 04516) have shown high levels of resistance to multiple isolates (Gaur and Gowda, 2005). BGM is another major foliar disease of chickpea (Pande *et al*., 2006). A major constraint in breeding for BGM resistance is the non-availability of high level of resistance in chickpea germplasm. High level of resistance to BGM has been observed in wild *Cicer* species *C. echinospermum* and efforts are being made to utilize this species.

Pod borer (*Helicoverpa armigera* Hubner) is the most important pest of chickpea in all growing environments. Moderate levels of resistance is available in some germplasm

accessions (e.g. ICC 506 EB) of cultivated (Lateef and Pimbert, 1990), while higher levels of resistance were observed in some wild species (Sharma *et al*., 2005) and efforts are being made to combine the non-preference (antixenosis) mechanism of resistance identified in the cultigen (e.g. ICC 506 EB) and antibiosis mechanism of resistance identified in *C. reticulatum.*

Improving yield potential

Efforts are being made to develop genotypes with short internodes and erect growth habit as such plant type may resist excessive vegetative growth in high input conditions. Two brachytic mutants, one spontaneous (E100 YM) and one induced (JGM 1), with short internodes and compact growth habit have been used in ideotype breeding and promising progenies with compact growth habit have been obtained (Gaur *et al*., 2008a).

Labor-saving varieties

Mechanization would play a key role in modernization of chickpea production. The farmers need chickpea varieties which can be directly harvested by combine harvesters. Recent efforts in India through ICRISAT-NARS partnership have led to release of three machine harvestable cultivars, namely Dheera or NBeG 47 (ICCV 05106), Phule Vikram (ICCV 08108) and RVG 204 (ICCV 08102). Several other breeding lines are in pipeline for release.

Another labor-saving trait desired in chickpea is tolerance to post emergence herbicides. Weed management by herbicides will not only be economical but also facilitate no-till methods, which help preserve topsoil, and help in reducing drudgery on farm women. Genetic variability has been identified for herbicide tolerance in chickpea germplasm/breeding lines/cultivars (Gaur *et al*., 2013a) and efforts are being made to develop chickpea varieties with desired level of herbicide tolerance.

Pigeonpea

Pigeonpea or red gram [*Cajanus cajan (L.) Millspaugh*] is an important food legume of the semi-arid tropics of Asia, Africa and Americas. It occupies a prime niche in sustainable farming systems of smallholder rainfed farmers. The productivity of pigeonpea has remained low and stagnant over the last few decades, thus this prompted scientists to search for novel ways of crop improvement. To tackle this challenge, Pigeonpea breeding unit at ICRISAT working on number of innovative ideas like, development of CGMS hybrids with 30 to 40 % yield advantage over traditional varieties, development of photo insensitive super early maturing lines, introgression of cleistogamous flower structure to maintain genetic purity of elite lines, use of obcordate leaf shape as NEP to assess genetic purity of hybrid parental lines and development of disease resistant

hybrids and integrated breeding approaches.

Inadequate genomic resources together with narrow genetic diversity in cultivated pool caused serious impediment to applying genomics assisted breeding (GAB) in pigeonpea (Pazhamala *et al*., 2015). To overcome this, several research groups were engaged in developing genomic and genetic resources. As a result a number of traits associated markers have been developed e.g. fusarium wilt (FW), sterility mosaic diseases (SMD), fertility restoration, days to 50% flowering, growth habit etc. Marker-assisted backcrossing (MABC) is being utilized to introgress resistance to diseases (FW and SMD) in susceptible cultivars as well as for pyramiding superior alleles into a single cultivar. Additionally, trait mapping using bi-parental crosses and multi-parental crosses such as MAGIC and NAM populations are in progress which will provide additional loci for GAB in pigeonpea. For complex traits which are governed by many genes/minor QTLs, genomic selection (GS) has been planned to implement. In order to assist pigeonpea hybrid breeding markers for cytoplasmic male sterility and fertility restoration have been identified. Simple sequence repeat (SSR) markers based hybridity purity assessment kits have also developed. Above mentioned markers are in routine use of pigeonpea hybrid breeding and providing quick and accurate solutions to breeders (Saxena *et al*., 2015).

Super early Lines

Day neutral and photo insensitive elite lines which mature in less than 100 days are recently developed in pigeonpea. These lines provide number of opportunities like expansion of pigeonpea on non-traditional area like rice fallow, could fit the pigeonpea-wheat cropping system, contribute to reduce environmental degradation, attractive option to grow the crop on stored soil moisture, can escape diseases, drought and pod borer attack (Kumar *et al*., 2015a).

CGMS hybrids

Hybrid breeding technology has been successfully developed in pigeonpea. The male–sterile lines carrying A4 cytoplasm from *C.cajanifolius* (Saxena *et al*., 2005) exhibits perfect male-sterility with absolutely no pollen production. This system has been reported to be highly stable under diverse environments (Dalvi *et al*., 2008). The hybrids ICPH 2671, ICPH 3762 and ICPH 2740 were released in India for commercial cultivation by the farmers. These hybrids are resistant to major diseases in pigeonpea viz. fusarium wilt and sterility mosaic disease and possess yield advantage of 30 to 40 percent over varieties (Kumar 2015 *et al*., 2015b).

Obcordate hybrids

To develop a stable and robust hybrid seed production technology, a novel idea of incorporating naked eye polymorphic marker [NEP] of

obcordate leaf shape was introduced in already established male sterile lines by back crossing scheme to track the purity of female parental lines. Since obcordate leaf shape is governed by single recessing gene, all the hybrids will be having normal lanceolate leaves in Ax R hybrid seed production and obcordate leaf shape in A x B maintenance programme. ICPA 2203 and ICPA 2204 are identified as stable male sterile lines with good general combining ability and ICPL 20116 is identified as fertility restorer which yielded disease resistant high yielding hybrids (Patil *et al.,* 2014).

Cleistogamous trait

Pigeonpea is an often cross pollinated species and out crossing extent up to 25-30 % (Saxena *et al*., 1990) and it is considered to be a prime constraint in maintaining genetic purity of cultivars and genetic stocks . To maintain a variety true to type especially in partially out-crossed species, it needs lot of resources in terms of isolation distance, installation of insect proof cages and labor charges for rouging and seed cleaning operations. Considering these facts attention was paid on natural mutant with wrapped flower morphology or cleistogamy. Stable lines are developed with this trait and are being used in crop improvement (Kumar *et al.,* 2015c).

Groundnut

Groundnut or peanut, an important oilseed and food legume crop is cultivated in 25.44 million ha with 45.22 m tons of production (FAOSTAT, 2014), of which Africa and Asia account for 95% of area and 91% of production. Groundnut breeding programs in various countries and ICRISAT (International Crops Research Institute for Semi-Arid Tropics) have contributed to release varieties to meet the needs of the producers, processors, and consumers. ICRISAT has supported the groundnut breeding programs in several countries in Africa and Asia and the partnerships led to release of ca. 130 improved varieties in 38 different countries that contributed to enhanced production and productivity.

Groundnut is a self-pollinated crop and pedigree method of breeding has been the choice. At ICRISAT, the groundnut breeding pipeline has successfully adopted ‘single seed decent’ method of breeding to optimize resources and improve traits with low heritability. Phenotyping for various agronomic and quality traits, yield evaluation trials, and early generation testing in target sites have largely contributed to enhance selection efficiency in breeding and testing pipelines (Janila and Nigam 2012). With the advent of molecular markers, genomic tools have been deployed in breeding pipelines to enhance selection efficiency in early generations and optimize resources. More recently, market-traits such as, oil content, oleic acid content and blanchability are focused in addition to production traits to meet end-use needs and demand from food industry.

Marker Assisted Backcross (MABC) breeding was used to combine early maturity with foliar fungal disease resistance the resulted in selection of introgression lines with 39–79% higher mean pod yield and 25–89% higher mean haulm yield over their respective recurrent parents (Janila *et al*., 2016d). A major effect QTL region explaining 80% Phenotypic Variance (PV) for rust resistance and 68% for resistance was targeted following the MABC approach is given in Varshney *et al*. (2014d). Marker Assisted Selection (MAS) and MABC breeding approaches were used to breed 'high oleic' lines in the background of Spanish and Virginia bunch types (Janila *et al*., 2016c). Under HTGP (High throughput genotyping platform) project supported by BMGF, we are using SNP-based markers for cost-effective genotyping with a turnover time of two weeks. In 2017 we have genotyped 18,000 plants and another 10,000 will be genotyped by end of 2017 using 10-SNP panel for three traits, viz., high oleic, and resistance to LLS and rust diseases. As we embark upon large scale genotyping, field logistics for collection of leaf samples, decision support tools for selections based on genotype, and rapid generation advancement need to be put in place to achieve the expected gains.

Transgenics

ICRISAT has recognized importance of the application of the genetic engineering technologies for the genetic enhancement of its mandate grain legumes owing to their narrow gene pool. Extensive efforts have been made at ICRISAT to develop efficient and reproducible tissue culture and transformation systems for production of transgenic pulses (Dayal et al., 2003; Sharma *et al*., 2006, Bhatnagar-Mathur *et al*., 2009a&b). Tissue culture and transformation methods based on *Agrobacterium*-mediated gene transfer for groundnut, pigeonpea, and chickpea, are now available for routine applications.

The successes with genetic transformations have resulted in developing transgenic grain legumes carrying genes for resistance to insect pests, fungal pathogens, tolerance to drought stress and nutritional enhancement (Bhatnagar-Panwar *et al*., 2015). Since resistance to *Helicoverpa armigera,* or the legume pod borerin chickpea and pigeonpea germplasm has so far been found to be low to moderate, transgenic resistance using insecticidal genes has been developed to achieve sustainable levels ofresistance to this insect pest (Gopalaswamy *et al*., 2008). Transgenic progenies have been screened based on the molecular data, ELISA, and detached leaf and pod bioassays showing significant reduction in damage rate and larval weights in comparison to the untransformed controls. While several events using single *cry* gene have been selected for event selection trials under confined fields, a number of transgenic events have been developed using *Bt*

gene stacks for achieving durable resistance for this pest as well as the pod borer complex. For abiotic stress tolerance, particularly drought, genetically engineered desi-type chickpea has been engineered to ectopically overexpress an osmoregulatory gene *P5CSF129*A for the overproduction of proline (Bhatnagar-Mathur *et al*., 2009a) and At*DREB1A*, driven by the stress-inducible *rd29A* promoter (Anbazhagan *et al*., 2015). Four Transgenic events in advanced generations (T6) with single copies were evaluated under water stress in lysimeters in a biosafety greenhouse under progressive water stress. While, one event reduced its transpiration in drier soil and higher vapor pressure deficit (VPD) range (2.0–3.4 kPa), two of these showed increased biomass partitioning into shoot, denser rooting in deeper layers of soil profile and higher transpiration efficiency than the untransformed controls, indicating the implicit influence of *rd29A:DREB1A* on mechanisms underlying water uptake, stomatal response, transpiration efficiency and rooting architecture under water stress (Anbazhagan *et al*., 2014, 2015). Similarly, for inducing herbicide tolerance in chickpea, transgenic interventions have been made towards developing resistance to PSII targeting herbicides (unpublished data). Transgenic events of chickpea expressing P450 cytochrome genes from soybean and artichoke have been developed and screened for herbicide tolerance using pre-emergence herbicides such as Linuron and Chloroturon that are photosynthesis inhibitors. Several events have shown resistance against linuron and are being characterized in advanced generations. Gene expression studies with key genes involved in photosynthetic as well as antioxidative machinery indicated up regulation of APX and CBP genes in the transgenic events as compared to the controls. More recently, genome editing tools for large-scale genome engineering in legumes are being developed to accelerate trait development and understanding the gene functions and their interactions. These toolkits/ platforms are being developed for testing several key genes to reveal the efficiency of these systems in grain legumes. A repository of pre-integrated Cas9 lines is also under development for making these available to the larger research community for facilitating both forward and reverse genetics towards enhancing genetic gains in these pulse crops.

Groundnut transgenics have been developed for economically important viruses such as Indian Peanut Clump virus (IPCV), groundnut rosette assistor virus (GRAV), an important component of the virus complex causing groundnut rosette disease in sub-Saharan Africa, Tobacco Streak virus (TSV) and PBNV, Peanut Bud necrosis virus (Sharma and Anjaiah, 2000; Rao *et al*., 2013). Similarly, transgenic groundnut events expressing the rice chitinase gene

(Rchit) were generated and evaluated for their tolerance to foliar fungal diseases including LLS and rust. Overexpressed chitinases imparted enhanced protection by degrading the chitin in hyphae, thereby retarding fungal growth, and by releasing pathogen-borne elicitors that induced defense reaction in plants (Prasad *et al*., 2013). Besides, abiotic constraints like drought are being addressed in groundnut using rd29A driven DREB1A, where selected events have been comprehensively tested in green house and confined field trials in various water stress regimes under varying vapour pressure deficits (VPDs). Several transgenic events had significantly higher seed filling under drought and displayed 20–30 % lower pod yield reduction than their untransformed counterparts under drought stress without displaying any yield penalty under irrigated conditions (Bhatnagar-Mathur *et al*., 2007, 2009b, 2014).

To address more complex and important aspects like pre-harvest *Aspergillus flavus* infection and resulting aflatoxin contamination, ICRISAT has developed high level of resistance in groundnut by over expressing (OE) antifungal plant defensins and through host-induced gene silencing (HIGS) of aflatoxin biosynthetic pathway genes. While the former improves genetic resistance to *A. flavus* infection, the latter inhibits aflatoxin production in the event of infection providing durable resistance against different *Aspergillus flavus* morphotypes and negligible aflatoxin content in several groundnut events/ lines well (Sharma *et al*., unpublished results).

Microbiology

Use of secondary metabolites for promotion of plant growth, control of insect pests and plant pathogens, and biofortification

There is a growing interest in the use of secondary metabolites, such as toxins, proteins, hormones, vitamins, amino acids and antibiotics, from microorganisms, particularly from actinomycetes, for promotion of plant growth, control of insect pests and plant pathogens and biofortification as these are readily degradable, highly specific and less toxic to nature. Actinomycetes are a group of Gram-positive bacteria with high G + C content belonging to the order Actinomycetales, which form branched mycelium and hence have sometimes been classified as *fungi imperfecti*. These are found most common in soil and compost and play an important role in the decomposition of organic wastes and produce secondary metabolites of commercial interest. Actinomycetes isolated from compost and rhizosphere soil have been reported to promote plant growth and inhibit phytopathogens including *Sclerotium rolfsii, Fusarium*

oxysporum f. sp. *ciceri* (FOC), *Rhizoctonia bataticola*, *Macrophomina phaseolina* and insect pests including *Helicoverpa armigera* and *Spodoptera litura*. The Microbiology unit of ICRISAT has identified 26 bacteria (of which 19 were actinomycetes) that has been demonstrated under field conditions for their usefulness and mechanisms such as plant growth-promotion (PGP), biological control on both insect pests and pathogens and biofortification potentials. From these promising microbes, ICRISAT has also identified few novel secondary metabolites that are responsible for mortality/inhibition of insect pests and pathogens, which can be further exploited for PGP/biocontrol applications under on-farm conditions. The following are ICRISAT's contribution in the field of Microbiology:

Role of bacteria on plant growth promotion (PGP)

A total of 137 bacteria were screened for their antagonism against important fungal pathogens of chickpea such as *S. rolfsii*, FOC, *R. bataticola* and *M. phaseolina* by dual culture and metabolite production assays. Nineteen most promising actinomycetes and seven other bacteria were evaluated for their physiological and PGP properties under *in vitro* and *in vivo* conditions. All the isolates exhibited good growth at temperatures from 20─40^{o}C, pH range of 7─11 and NaCl concentrations up to 8 %. These were also found highly tolerant to Bavistin, slightly tolerant to Thiram and Captan but susceptible to Benlate and Ridomil at field application levels and were found to produce siderophore, cellulase, lipase, protease, chitinase, hydrocyanic acid, indole acetic acid (IAA) and β-1,3-glucanase. When these actinomycetes and other bacteria were evaluated for their PGP properties under field conditions on chickpea, all exhibited increase in nodule number (up to 25%), shoot weight (up to 20%) and yield (up to 16%). The actinomycetes treated plots enhanced total N (up to 15%), available P (up to 18%) and organic C (up to 15%) over the un-inoculated control plots. The scanning electron microscope (SEM) studies exhibited extensive colonization by actinomycetes and bacteria on the root surface of chickpea. The expression profiles for IAA, siderophore and β-1,3-glucanase genes exhibited up-regulation for all three traits. The actinomycetes were identified as *Streptomyces* but different species in the 16S rDNA analysis. It was concluded that the selected actinomycetes have good PGP potentials on chickpea. The actinomycetes and bacteria were also demonstrated for their PGP potentials on other crops including pigeonpea, rice and sorghum (Gopalakrishnan *et al*., 2012a; 2012b; 2013a; 2013b; 2014a; 2014b; 2015a; 2015b; 2016a; 2016b; Sreevidya *et al*., 2016a).

Role of bacteria and their metabolites on biological control of insect pests

Helicoverpa armigera (Hübner) and *Spodoptera litura* (Fabricius) are an important insect pests causes serious damage to grain legumes. Microorganisms produce a range of metabolites with varying pest control properties. With this concept in mind, we had identified 15 *Streptomyces* strains with insecticidal activity against *H. armigera* and *S. litura*.Among the 15 isolates identified, SAI-25 (*Streptomyces griseoplanus*), CAI-155 (*Streptomyces sp.*) and BCA-698 (*Streptomyces sp.*) were found to be promising candidates as broad-spectrum biocontrol agents. We evaluated an insecticidal compound purified from the extracellular extract of *S. griseoplanus* SAI-25 by bioactivity guided fractionation against *H. armigera*. Spectral studies confirmed that the purified compound was cyclo(Trp-Phe) of the diketopiperazines class. Cyclo(Trp-Phe) exhibited antifeedant (70%), larvicidal (67%) and pupicidal (59%) action against *H. armigera* in a dose-dependent manner. The LD_{50} and LD_{90} values for larvicidal effect were 619 and 2750 ppm, respectively. In addition, the purified compound prolonged larval (10.3-11.1 days) and pupal (10.9-11.8 days) periods as compared to the untreated control (larval duration - 9.8 days; pupal duration - 10.6 days). This is the first report on the presence and biological activity of cyclo (Trp-Phe) isolated from the genus *Streptomyces*. One more metabolite was also purified from the culture filtrate of *Streptomyces* sp. CAI-155. The culture filtrate of CAI-155 was extracted using Diaion HP-20 and the active fractions were fractionated on Silica and C18 column chromatography. The C18 active fraction was further fractionated on Silica gel 60 F_{254} thin layer chromatography (TLC). The most active fraction (Rf 0.64) purified from TLC led to the identification of a novel metabolite *N*-(1-(2,2-dimethyl-5-undecyl-1,3-dioxolan-4-yl)-2-hydroxyethyl)stearamide by spectral studies. The purified metabolite showed 70─78% mortality in 2nd instar *H. armigera* by diet impregnation assay, detached leaf assay and greenhouse assay. The LD_{50} and LD_{90} values of the purified metabolite were 627 ppm and 2276 ppm, respectively. Both of these novel metabolites can be exploited for pest management in future (Gopalakrishnan et al. 2016a; Sathya *et al*., 2016a; Vijayabharathi *et al*., 2014).

Role of bacteria and their metabolites on biological control of plant pathogens

A total of 137 actinomycetes, isolated from 25 different herbal vermin-composts, were characterized for their antagonistic potential against FOC by dual-culture assay. Of them, five most promising FOC antagonistic isolates (CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90) were further

characterized for antagonistic potential against *Macrophomina phaseolina* (*Rhizoctonia bataticola*), which causes dry root rot in chickpea and sorghum. In the dual-culture assay, three of the FOC antagonistic isolates, CAI-24, KAI-32 and KAI-90, inhibited *R. bataticola*, while two of them inhibited *M. phaseolina* (KAI-32 and KAI-90). When the FOC antagonistic isolates were evaluated further for their antagonistic potential in the greenhouse and wilt-sick field conditions on chickpea, 45−76% and 4−19% reduction of disease incidence were observed, respectively, over the control. The sequences of 16S rDNA gene of the isolates CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90 were matched with *Streptomyces tsusimaensis, S. caviscabies, S. setonii, S. africanus* and *Streptomyces* spp., respectively, in the BLAST analysis. This study indicated that the selected actinomycete isolates have the potential for biological control of *Fusarium* wilt disease in chickpea (Gopalakrishnan *et al*., 2011).

In a study against *Botrytis cinerea*, causing Botrytis Gray Mold (BGM) disease in chickpea, ten bacteria and one fungus were found promising. The culture filtrate of the most promising isolate, VFI-51, was further purified by various chromatographic techniques and identified as "citrinin" by spectral studies. The efficacy of citrinin was demonstrated to control BGM in chickpea under greenhouse conditions. The sequences of 18S rDNA gene of the VFI-51 matched with *Penicillium citrinum* in BLAST analysis. Under greenhouse and field conditions, VFI-51 significantly enhanced the nodule number, nodule weight, rootand shoot weight and stover and grain yield over the un-inoculated control. In the rhizosphere, VFI-51 also significantly enhanced total N, available P and OC over the un-inoculated control. Scanning electron microscopy analysis revealed that VFI-51 colonized on the roots of chickpea. This study concluded that VFI-51 have the potential for biocontrol of BGM and plant growth promotion in chickpea. VFI-51 was also demonstrated to have antagonistic potential against charcoal rot disease in sorghum (Sreevidya *et al*., 2015, Sreevidya and Gopalakrishnan, 2016b).

Role of bacteria on biofortification

A study was done to test the potential of plant growth-promoting actinobacteria in increasing seed mineral density of chickpea under field conditions. Among the 19 isolates of actinobacteria tested, significant ($p< 0.05$) increase of minerals over the un-inoculated control treatments was noticed on all the isolates for Fe (10─38%), 17 for Zn (13─30%), 16 for Ca (14─26%), 9 for Cu (11─54%) and 10 for Mn (18─35%) and Mg (14─21%). The increase might be due to the production of siderophore producing capacity of tested actinobacteria, which was confirmed in our previous

studies by q-RT PCR on siderophore genes expressed up to 1.4 to 25 fold increased relative transcription levels. The chickpea seeds were subjected to processing to increase the mineral availability during consumption. The processed seeds were found to meet recommended daily intake of FDA by 24─28% for Fe, 25─28% for Zn, 28─35% for Cu, 12─14% for Ca, 160─167% for Mn and 34─37% for Mg. It is suggested that, microbial inoculum can serve as a complementary sustainable tool for the existing biofortification strategies and substantially reduces the chemical fertilizer inputs (Sathya et al. 2016b).

Concluding Remarks

Recent years have witnessed impressive progress in research and development of grain legumes. Impressive yield growth has been seen in some countries where adoption of improved cultivars and production technologies was high. The integration of novel tools and techniques provides opportunity for accelerating process of development of improved cultivars. Enhanced investments are needed in research on grain legumes and developmental activities for enhancing adoption of improved cultivars and technologies. In addition to increasing productivity of grain legumes, other aspects of increasing profitability to farmers from grain legumes, such as enhancing mechanization, reducing post-harvest losses, developing value-added products and linking to markets, should be considered.

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