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# Translational genomics and molecular breeding for enhancing precision and efficiency in crop improvement programs: Some examples in legumes

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**Abstract**

**Legumes like chickpea, pigeonpea and groundnut are protein rich, nutrient-dense, and nitrogen fixing crops. Their importance is increasingly recognized in view of the urgent need to address burgeoning malnutrition problem and to impart sustainability to cropping systems. Breeding programs in these crops have achieved great success. However, consistent improvement in genetic gains demands integration of innovative tools and technologies with crop breeding programs. Genomic resources are of paramount significance in context of improving the efficiency and precision of crop breeding schemes. The last decade has witnessed a remarkable success in generating unprecedented genomic resources in these crops, thus transforming these genomic orphans into genomic resource rich crops. These genomic resources include array-based genotyping platforms, high-resolution genetic linkage maps/HapMaps, comprehensive transcriptome assemblies and gene expression atlas, and whole genome sequences etc. Further progression from the training phase (development) to breeding (deployment) phase is marked with the current availability of a variety of molecular breeding products in these legume crops. In the present review, we discuss how deployment of the modern genomic resources such as next-generation gene discovery techniques and "gold standard experimental designs" is furthering our knowledge about the genetic underpinnings of trait variation. Also, key success stories demonstrating the power of molecular breeding in these legume crops are highlighted. It is opined that the breeding populations constantly improved by sequence-based breeding approach will greatly help improving breeding traits and the genetic gains accruable from crop breeding programs.**

**Key words:** Legume, DNA markers, gene, genome, trait mapping

## Introduction

Legume crops are important in terms of nutritional security owing to their high protein and nutrient contents (Bohra et al. 2015; Varshney et al. 2015, 2018). The signature features of these crops such as biological nitrogen fixation contribute greatly to sustainable cropping systems. Improving these crops with classical breeding tools has made significant progress, with development and release of a number of varieties in these crops that suit a range of agro-ecologies in India. For instance, more than 190 chickpea varieties (both state and centrally released) have been developed over the last five decades for cultivation across diverse agro-ecological zones in India. A quantum leap has been witnessed in pulses production in recent years (http://agricoop.gov.in/sites/default/files/1stadvest_201819E.pdf) and a record production of pulses (25.23 mt) reported during year 2017-18 reflects that self-sufficiency has been achieved in terms of production of these protein-rich food crops in India. However, burgeoning population and increasing malnutrition problem demand genetic gains accrued from crop breeding programs to improve perpetually and increasingly. In the context, genome tools and technologies made available in recent years hold promise in enhancing breeding efficiency and genetic gains per unit time. Recent advances in next generation sequencing (NGS) technologies have dramatically impacted upon the legume genomics, leading to the development of a variety of genomic tools and technologies including the whole genome

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sequence in these crops (Varshney et al. 2013a, 2015). Deployment of genomic tools in these crops has delivered a series of molecular breeding crop products for future cultivation in farmers' field (Varshney et al. 2018). Here we review the status of the availability of genomic tools in the three major legume crops i.e. chickpea, pigeonpea and groundnut (an important oilseed crop), and highlight key examples of molecular breeding in the select crops. We underscore the role of sequence-based breeding for improving genetic gains in these crops. We also highlight future opportunities and challenges that lie ahead while embracing these modern genomic tools and technologies for accelerating crop improvement.

## Modern genomic recourses in the post-genome sequencing era

Significant achievements have been made in these crops over the last decade in terms of generation of a variety of genomic recourses. In the following section, we briefly discuss about the large-scale genomic tools developed recently in these crops.

### *Genome-wide DNA markers*

The first sets of large-scale DNA markers in these crops were reported in the form of simple sequence repeat (SSR) markers developed from BAC-end sequences (BESs) (Bohra et al. 2011; Thudi et al. 2011). Increasing adoption of high throughput next generation sequencing (NGS) technologies has further leveraged the arsenal of genetic markers in legume crops, particularly single nucleotide polymorphism (SNP) markers for genotyping applications. Initial examples of genome wide SNP discovery include 2,486 SNPs in chickpea (Hiremath et al. 2012), 1,616 SNPs in pigeonpea (Saxena et al. 2012) and 53,257 SNPs in groundnut (Zhou et al. 2014). Similarly, genome-wide DNA markers including 1,19,169 and 1,10,491 intron-spanning markers (ISM) from 23,129 desi and 20,386 kabuli protein-coding genes and 7,454 *in silico* InDel markers from 3283 genes were developed in chickpea (Srivastava et al. 2016). More recently, analysis of whole genome resequencing (WGRS) data in these crops has facilitated construction of high throughput SNP genotyping platforms referred to as SNP chips. For instance, 50,590 SNPs were tiled on 'Axiom®*CicerSNP* Array after extracting high quality nonredundant SNPs from the resequencing data of 429 chickpea lines (Roorkiwal et al. 2018a). Similarly, a total of 58,233 high-quality SNPs identified from sequencing/RNA seq data of 41 genotypes (30 tetraploids and 11 diploids) were tiled on 'Axiom_Arachis' 50K array in groundnut (Pandey et al. 2017a). In pigeonpea, Axiom *Cajanus* SNP array was developed with 56,512 unique and informative sequence variations from the WGRS data of 104 pigeonpea lines (Saxena et al. 2018).

### *High-density genetic maps*

Genome mapping is key to delineate the specific genomic regions that exert influence on the phenotypes of agricultural significance. Initial discovery of SNP markers was followed by adoption of automated platforms for SNP typing such as GoldenGate assay, VeraCode assay and more customized Kompetitive Allele Specific PCR (KASP) assay (Hiremath et al. 2012; Saxena et al. 2012; Deokar et al. 2014; Gaur et al. 2015). In recent years, application of sequence-based genotyping assays such as genotyping-by-sequencing (GBS) (Saxena et al. 2017a, b, c), restriction-site associated DNA sequencing (RAD-seq) (Zhou et al. 2014), specific length amplified fragment (SLAF) sequencing (Hu et al. 2018) etc. that allow rapid discovery and mapping of thousands of loci has caused a marked increase in the number of molecular markers, thus dramatically improving the marker density or resolution of the current genetic linkage maps in these crops.

Since reference genomes are now available in these crops, low-depth WGRS also referred to as skim sequencing is emerging as a cost-efficient and accurate tool for high-throughput genotyping while overcoming the inherent drawbacks of GBS technology such as missing data and ascertainment bias. For instance, Kale el al. (2015) applied skim sequencing approach in chickpea for analyzing 232 recombinant inbreds and the parental genotypes. The mapping parents ICC 4958 and ICC 1882 were sequenced with an estimated 8× coverage, while the RILs were sequenced at an average depth of 0.72X. A total of 53,223 SNPs could be placed into 1,610 bins onto eight chickpea pseudomolecules following a parent dependent sliding window approach. Table 1 provides a non-exhaustive list of high-density genetic maps developed in the three legume crops. Latest additions to this include 13, 679- and 7769- SNP loci genetic linkage maps with 1033.67 cM and 1076.35 cM length, respectively of the two RIL populations (ICC 4958 × ICC 1882 and ICC 283 × ICC 8261) (Roorkiwal et al. 2018a). Apart from enabling better prioritization of the candidate genes and fine mapping, these highly saturated genetic maps are greatly helpful in anchoring

**Table 1.** List of some high-density genetic linkage maps in three legume crops

| Crop | Mapping Population (Type) | Number and type of markers | Map length (cM) | Reference |
|---|---|---|---|---|
| Chickpea | ICC 4958 × PI 489777 (RIL) | 1,328 (SNP, SSR, DArT and CISR) | 788.6 | Hiremath et al. (2012) |
| | ICC 4958 × ICC 17160 (RIL) | 8,34 (SSR and SNP) | 949.4 | Saxena et al. (2014) |
| | ICC 12299 × ICC 8261 (RIL) | 3,625 (SNP) | 714.1 | Kujur et al. (2015) |
| | ICC 4958 × PI 489777 (RIL) | 6,698 (SNP, SSR and others) | 1,083.9 | Gaur et al. (2015) |
| | ICC 4958 × ICC 1882 (RIL) | 13, 679 (SNP) | 1,033.7 | Roorkiwal et al. (2018a) |
| | ICC 283 × ICC 8261 (RIL) | 7,769 (SNP) | 1,076.4 | Roorkiwal et al. (2018a) |
| Pigeonpea | ICP 28 × ICPW 94 (F2) | 910 (SNP, SSR) | 996.21 | Saxena et al. (2012) |
| | Asha ×UPAS 120 (F2) | 932 (SSR, SNP) | 1,411.8 | Arora et al. (2017) |
| | Pusa Dwarf × H2001-4 (F2) | | | |
| | Pusa Dwarf × HDM04-1 (F2) | | | |
| | ICPL 20096 × ICPL 332 (RIL) | 1,101 (SNP) | 921.21 | Saxena et al. (2017a) |
| | ICP 8863 × ICPL 87119 (F2) | 996 (SNP) | 1,597.3 | Saxena et al. (2017a) |
| | ICPB 2049 × ICPL 99050 (RIL) | 964 (SNP) | 1,120.6 | Saxena et al. (2017b) |
| | ICP 5529 × ICP 11605 (F2) | 787 (SNP) | 1,454 | Saxena et al. (2017c) |
| Groundnut | PI 475887 × Grif 15036 (F2) | 1,724 (SNP, SSR) | 1,081.3 | Nagy et al. (2012) |
| | Zhonghua 5 × ICGV86699 (RIL) | 1,685 (SNP, SSR) | 1,446.7 | Zhou et al. (2014) |
| | Zhonghua 10 × ICG 12625 (RIL) | 1,219 (SSR) | 2,038.7 | Huang et al. (2016) |
| | ICGV 00350 × ICGV 97045 (F2) | 1,152 (DArT/DArT-seq) | 2423.1 | Vishwakarma et al. (2016) |
| | ICGV 07368 × ICGV 06420 (F2) | 854 (DArT/DArT-seq) | 3,526 | Shasidhar et al. (2017) |
| | ICGV 06420 × SunOleic 95R (F2) | 1,435 (DArT/DArT-seq) | 1,869 | Shasidhar et al. (2017) |
| | Huayu28 X P76 (RIL) | 2,334 (SNP, SSR) | 2,586.3 | Hu et al. (2018) |
| | ZH16 × sd-H1 (RIL) | 3,630 (SNP) | 2,098.1 | Wang et al. (2018) |

of scaffolds from the draft genome sequences (Gaur et al. 2015).

***Compressive transcriptome assemblies and gene expression atlas***

Considerable research has been invested to understand the plant's response to various biotic and abiotic stresses and corresponding alterations in the gene expression patterns, even before the whole genome sequencing of these crops (see Varshney et al. 2015). The initial studies on gene expression, based on expressed sequence tags (ESTs) and microarrays, have contributed significant functional genomic resources for gene discovery and marker development in these crops (Mantri et al. 2007; Varshney et al. 2009; Raju et al. 2010). A recent shift from low/moderate scale expression profiling (Sanger ESTs/microarrays) to large-scale digital gene expression (DGE) analysis has facilitated a global view on the transcriptional dynamics that underlies plant growth and development and response to a variety of stresses.

Apart from enabling isolation /cloning of important genes such as *CarNAC3* (Peng et al. 2009), *CarF -box1* (Jia et al. 2012) in chickpea and *CcCYP* in pigeonpea (Sekhar et al. 2010), the transcriptomic resources have led to the development of gene-based marker systems in these crops. For example, Hiremath et al. (2011) reported 26, 252 SSRs and 26, 082 SNPs from transcriptome assembly in chickpea. Similarly, 50,566 SSRs and 12,141 SNPs were

**Table 2.** Some next generation trait mapping studies in three legume crops

| Crop | Trait | Mapping population | Population size | QTL/candidate genes | $R^2$ (%) | Candidate genomic region (Chromsome/LG) | Reference |
|---|---|---|---|---|---|---|---|
| Chickpea | 100-seed | ICC 7184 x ICC15061 (RIL) | 221 | CaqSW1.1/Six genes including constitutive photomorphogenic9 (COP9) signalosome complex subunit 8 (CSN8) gene | 47.60 | 35 kb (Chromosome 1) | Das et al. (2015) |
| | 100-seed weight | ICC 4958 x ICC 1882 (RIL) | | Five genes including Ca_04364 and Ca_04607 | 28.61 and 19.25 | 1.08 Mb (CaLG01) and 2.7 Mb (CaLG04) | Singh et al. (2016a) |
| | Total dry root wt. to total plant dry wt. ratio | | | Four genes including Ca_04586 | 23.39 and 24.46 | 1.10 Mb (CaLG04) | |
| | Flowering time | ICC 4958 x ICC 17163 (RIL) | 260 | CaqaDTF4.1, CaqaDTF4.2, Caqb DTF4.1, CaqbDTF4.2/efl1 and GI | 33-49 | 757-kb and 907.1-kb (Chromosome 4) | Srivastava et al. (2017) |
| | | ICC 4958 x ICC 8261 (RIL) | 204 | | | | |
| | *Ascochyta* blight | FLIP84-92Cx PI359075 (RIL) | 250 | qABR4.1/CaAHL18 gene | - | 500 kb (Chromosome 4) | Kumar et al. (2018) |
| | | FLIP84-92C x PI599072 (RIL) | 217 | | | | |
| Pigeonpea | *Fusarium* wilt (FW) | ICPL 20096 x ICPL 332 (RIL) | 188 | Four candidate genes including C. cajan_03203 | | CcLG02 and CcLG11 | Singh et al. (2016b) |
| | | | | Three InDels | | CcLG02, CcLG07 and CcLG08 | Singh et al. (2017) |
| | Sterility mosaic disease (SMD) | ICPL 20096 x ICPL 332 (RIL) | | Three candidate genes including C. cajan_01839 | | CcLG02, CcLG08 and CcLG11 | Singh et al. (2016b) |
| | | | | Two InDels | | CcLG02 and CcLG10 | Singh et al. (2017) |
| Groundnut | Rust resistance | TAG 24 x GPBD 4 (RIL) | | 25 candidate genes | 42.7-83.6 | 3.06 Mb (A03) | Pandey et al. (2017b) |
| | Late leaf spot | | | Nine candidate genes | 9-63.1 | 2.98 Mb (A03) | |
| | Late leaf spot | Florida-07 x GP-NC WS 16 (RIL) | 192 | - | - | 4.7 Mb (A05), 1.2 Mb (B03) and 3.4 Mb (B05) | Clevenger et al. (2018) |
| | Shelling %age | Yuanza 9102 x Xuzhou 68-4 (RIL) | 195 | Nine candidate genes | 8.18-20.26 | 2.75Mb (A09), 1.1Mb (B02) | Luo et al. (2018) |

reported in pigeonpea following survey of a transcriptome assembly (Dubey et al. 2011). The most comprehensive transcriptome assembly in pigeonpea combines sequence data (Illumina, 454 and Sanger ESTs) from >16 genotypes (Kudapa et al. 2012) and 6,284 intron spanning region (ISR) markers were reported.

Thousands of differentially expressed genes (DEGs) emanating from gene expression experiments still remain inadequate to explain translation of genome sequence information into plant phenotypes (Kudapa et al. 2018). To bridge this gap, *Cicer arietinum* gene expression atlas (CaGEA) was constructed from deep sequencing of 27 samples from five developmental stages (germination and seedling, vegetative, reproductive, and senescence) of the drought tolerant chickpea ICC 4958. The CaGEA uncovered 15,947 unique DEGs and gene clusters involved in growth and development and importantly, nine differentially expressed *QTL-hotspot* genes (inferred from RNA Seq) were validated through qRT-PCR. Similarly, the *Cajanus cajan* gene expression atlas (CcGEA) based on the RNA seq data from 30 samples spanning five stages of the genotype Asha, and the CcGEA revealed a total of 28,793 significantly expressed genes, with 28 flowering related genes and three hub genes playing key role in pollen development and seed formation (Pazhamala et al. 2017). The groundnut gene expression atlas covering 22 different tissue types profiled genes and gene networks that control growth and development such as flowering and geocarpy (Clevenger et al. 2016; http://bar.utoronto.ca/efp_arachis/cgi-bin/efpWeb.cgi). Also, significance of alternative splicing events and non-coding RNAs was also discussed in the developmental context. The gene networks comprised of genes involved in vegetative, reproductive and seed development (Clevenger et al. 2016). Another gene expression atlas for groundnut is underway at ICRISAT (unpublished). The global view of gene expression patterns as elucidated by these comprehensive transcriptomic resources will greatly support basic and applied research for crop improvement in legumes.

### *Whole genome sequencing*

The reference genome sequences are now available in all these three crops, thanks to the appearance and subsequent democratization of NGS technologies (Bohra and Singh 2015, Varshney et al. 2013a). Draft whole genome assemblies of ~738-Mb and ~605-Mb were reported for kabuli chickpea genotype CDC Frontier (Varshney et al. 2013b) and a popular pigeonpea variety Asha (ICPL 87119) (Varshney et al. 2012) respectively. Given the allotetraploid nature of cultivated groundnut, Bertioli et al. (2016) assembled 1,211-Mb and 1,512-Mb genomes of its diploid ancestors *Arachis duranensis* and *Arachis ipaensis*, respectively. Concerning the salient features of these genome assemblies, chickpea genome assembly contains 28,269 genes and a GC content of 30.7%, while pigeonpea genome assembly has 48,680 genes and 32.8% GC content. The genome assemblies of *A. duranensis* and *A. ipaensis* contain 36,734 and 41,840 genes, respectively. Chen et al. (2016) assembled 1,051-Mb of genome of the A-genome progenitor *A. duranensis* containing 50,324 protein-coding gene and 31.8% GC content. These genome assemblies shed new light on the genomic regions related to breeding traits like disease resistance in chickpea (187 candidate genes) and groundnut (345 and 397 genes in the *A. duranensis* and *A. ipaensis* assembly, respectively), drought tolerance (111 candidate genes) in pigeonpea, and oil biosynthesis and allergens in groundnut (1,671 genes).

## Genetic landscape of important traits: Shifting paradigms and improved understanding of trait architectures

Determination of the genomic regions or DNA markers that explain substantial portion of the phenotypic variation for a given trait is of paramount significance in crop improvement. Classical QTL analysis using bi-parental populations has revealed several genomic regions/DNA markers associated with a variety of important traits in these crops. Some of the important traits that have been dissected using QTL mapping include stress resistance [Fusarium wilt (FW), ascochyta blight (AB), botrytis gray mold, and drought in chickpea; FW and sterility mosaic disease (SMD) in pigeonpea; and root knot nematode, rust, late leaf spot (LLS), rosette disease, tomato spotted wilt virus, and drought in groundnut] and other important traits such as double podding in chickpea, fertility restoration in pigeonpea and oil quality in ground nut (see Varshney et al. 2013a, 2015). However, classical QTL analysis remains time-consuming and labour-intensive. Integration of NGS with the gene mapping methods is greatly reducing the time, labour and cost that are otherwise invested in marker discovery and mapping in classical methods.

### *Rapid gene discovery in bi-parental populations*

Researchers in legume crops are now widely

embracing these next generation QTL mapping techniques such as QTL-seq for rapid discovery of the QTL/genes. QTL-seq combines bulked segregants analysis (BSA)/selective DNA pooling with WGRS to compute genome-wide SNP-index to delineate genomic regions influencing trait variation (Takagi et al. 2013). As shown in Table 2, RIL populations have been assayed with QTL-seq in legume crops to dissect agronomically important traits such as 100 seed weight (Das et al. 2015, Singh et al. 2016a), flowering time (Srivastava et al. 2017) and AB resistance (Kumar et al. 2018) in chickpea; resistance to FW and SMD (Singh et al. 2016b, 2017) in pigeonpea; and rust (Pandey et al. 2017) and LLS resistance (Pandey et al. 2017, Clevenger et al. 2018), and shelling percentage in groundnut (Clevenger et al. 2018). While analyzing 100-seed weight in chickpea, Das et al. (2015) narrowed down the candidate genomic region underlying the QTL 'CaqSW1.1' to 35 kb on chromosome 1 and identified six genes including constitutive photomorphogenic9 (COP9) signalosome complex subunit 8 (CSN8) gene. Similarly, four candidate genes including *C.cajan_03203* and three candidate genes including *C.cajan_01839* were identified in pigeonpea for resistance to FW and SMD, respectively by using Seq-BSA along with WGRS of

**Table 3.** QTL discovery and introgression in legume crops

| Crop | Trait | QTL Name | QTL mapping | MAS/MABC |
|---|---|---|---|---|
| Chickpea | Drought tolerance | *QTL-hotspot* | Varshney et al. (2014a) | TAA170, ICCM0249, and STMS11 (Varshney et al. 2013c) |
| | *Ascochyta* blight resistance | QTLAR1 | Iruela et al. (2006); Madrid et al. (2013) | CaETR (Bouhadida et al. 2013) GAA47, SCY17, TA130, TA2 (Varshney et al. 2014b) |
| | | QTLAR2 | Iruela et al. (2006) | SCY17590 (Bouhadida et al. (2013) GAA47, SCY17, TA130, TA2 (Varshney et al. 2014a) |
| | | $QTL_{AR3}$ | Iruela et al. (2007) | GA16, TS82, TA194, TR58 |
| | *Fusarium* wilt resistance | Foc1 | Mayer et al. (1997); Gowda et al. (2009), Sabbavarapu et al. (2013) | GA16, TAA60, TA194, TS82, TA110, TR19 (Varshney et a. 2014a) |
| | | Foc2 | Gowda et al. (2009) | TA 37, TA110 (Pratap et al. 2017) |
| | | Foc3 | Sharma et al. (2004); Gowda et al. (2009) | GA16, TAA60, TA194, TS82, TA110, TR19 (Varshney et a. 2014a) |
| | | Foc4 | Tullu et al. (1998, 1999) | GA16, TA59, TA96, TR19, TA27 (Mannur et al. 2019) |
| Groundnut | Rust resistance | QTLrust01 | Khedikar et al. (2010) | IPAHM103 (Varshney et al. 2014c) |
| | | QTLR4-rust01/ QTLR5-rust01 | Sujay et al. (2012) | GM1536 (Varshney et al. 2014c) |
| | | QTLR4-rust02 | Sujay et al. (2012) | GM2301, GM2079 (Varshney et al. 2014c) |
| | Nematode resistance | Rma | Chu et al. (2007a) Nagy et al. 2010 | SR 197, CAPS 1169/1170 (Chu et al. 2011) GM565 (Chu et al. 2011) |
| | Improved oil quality | ahFAD2A | Chu et al. (2007b); Chen et al. (2010) Chu et al. (2011); AS-PCR, CAPS Janila et al. (2016a; Bera et al. 2018) | CAPS 1101/1048 and a HybProbe SNP assay |
| | | ahFAD2B | Chu et al. (2009); Chen et al. (2010) | HybProbe SNP assay (Chu et al. 2011); AS-PCR, CAPS (Janila et al. 2016a; Bera et al. 2018) |
| | Late leaf spot | QTLLLS01 | Sujay et al. (2012) | Varshney et al. 2014b; Janila et al. 2016b |

four additional resistance and susceptible genotypes (Singh et al. 2016b). The same group applied InDel Seq approach in pigeonpea to identify InDels associated with the candidate genes underlying FW and SMD resistance (Singh et al. 2017). This facilitated identification of 16 candidate InDels, of which five were successfully validated. Importantly, the candidate genes identified through QTL-seq experiments were further validated by other approaches like classical QTL mapping, expression analysis and amplicon sequencing of the candidate genes in contrasting accessions/parental genotypes. Most recently, Luo et al. (2018) have identified nine candidate genes on chromosomes A09 and B02 for shelling percentage in ground nut, and further QTL analysis with the KASP markers developed targeting four ns SNPs could account nearly 20% variation to the two genomic regions. Takagi et al. (2013) have emphasized the relevance of QTL-seq in analyzing the experimental populations that are derived from closely related individuals, and its ability to fine dissect the genetic make up of natural quantitative variation unlike artificially-mutagenized traits in case of MutMap. This assumes significance for genetic analysis of experimental populations in legume crops where low level of genetic polymorphism in the cultivated pool is evident from considerable body of literature.

### *Genome wide association studies (GWAS)*

The GWAS has become a routine genomic tool to elucidate the genetic landscape of complex traits in diverse individuals assayed with genome-wide sets of genetic markers (Liu and Yan 2019). With more than 1000 studies reported over the last decades in different crops (Liu and Yan 2019), the GWAS is increasingly deployed to discover new genotype-phenotype associations for important traits in legume crops. In chickpea, the GWAS has revealed marker-trait associations (MTAs) for biotic stresses such as AB (Li et al. 2017), abiotic stresses like heat and drought stress related traits (312 MTAs; Thudi et al. 2014), and protein content (seven SNP loci; Upadhyaya et al. 2016a) and seed iron and zinc content (16 genomic loci/genes; Upadhyaya et al. 2016b) etc. In groundnut, GWAS of 158 groundnut accessions with 17,338 SNPs led authors to identify 41 MTAs for 11 domestication related traits, and the authors suggested selection sweeps on chromosome A3 based on the presence of 662 genes on this particular chromosome (Zhang et al. 2017). Earlier, Pandey et al. (2014) analyzed 300 accessions of reference set with 154 SSR and 4,597 diversity arrays technology (DArT) markers and detected a total of 524 MTAs with PV ranging between 5.81-90.09% for 36 important agronomic, disease and quality traits.

In view of the declining sequencing cost, the GWAS is combined with WGRS data using SUPER GWAS method for high-resolution trait mapping. For example, GWAS with WGRS data of chickpea accessions revealed 100-kb (AB4.1 QTL) and 437-kb regions on chromosome 4 for AB resistance (Li et al. 2017) and yield-related traits (Li et al. 2018), respectively. Similarly in pigeonpea WGRS data of 292 pigeonpea accessions facilitated identification of 241 MTAs, with CcLG09 carrying 90% of the MTAs detected for days to 50% flowering and six structural variations (SVs) explaining 75% of these MTAs. The study highlighted the important role of CcLG09 during the pigeonpea domestication and breeding (Varshney et al. 2017). The constantly decreasing cost of sequencing and the concurrent refinements in informatics tools will further motivate researchers to combine WGRS and GWAS to rapidly deliver the functional markers for genomics-assisted breeding in the legume crops.

### *Multi-parental mapping resources for enhanced trait dissection*

The “gold standard experimental designs” with balanced genetic structure and power of controlled crosses have been recently used in molecular mapping of complex traits in various crop plants like rice, wheat, maize etc. (see Wallace et al. 2012). Two of such designs are multiparental advanced generation intercross (MAGIC) and nested association mapping (NAM). These designs remain extremely relevant while dissecting the genetics of adaptation traits that are often confounded with the population structure (Wallace et al. 2012), and also for detection of epistatic interactions (Liu and Yan 2019). These designs allow incorporation of multiple founders and occurrence of profuse recombinational events. The multiparent mating designs MAGIC and NAM have been recently implicated in chickpea and pigeonpea. In chickpea, founder parents and 1,000 F6 MAGIC lines have been sequenced at depths of 10X and 2-3X, respectively and this sequencing data together with the phenotypic data will be used to conduct GWAS in this population (Huang et al. 2015). Availability of such high-power mapping resources will greatly facilitate enhanced trait discovery apart from broadening the genetic base of the current breeding programs of the legume crops.

### *Marker assisted selection (MAS) in legume crops: Notable Examples*

In legume crops, marker-assisted back crossing (MABC), the first generation molecular breeding tool, has been extremely successful in transferring QTL having large effects on the phenotype (Table 3). A recent report on MABC demonstrates fast-track transfer of wilt resistance in the background of FW (race 4)-susceptible chickpea cultivars Annigeri 1 and JG 74 (Mannur et al. 2019). The SSR-guided transfer of the QTL controlling FW resistance was accomplished using WR 315 (a resistant landrace) as the donor parent. In addition to the enhanced level of disease resistance, the improved versions thus obtained have also shown substantial yield advantage (8 to 53%) over the recurrent parents. Other notable examples of MABC in chickpea include introgression of *QTL-hotspot* into the background of an elite cultivar JG 11 (Varshney et al. 2013c), development of FW- and AB-resistant versions of the cultivar C 214 (Varshney et al. 2014b) and improved Pusa 256 having enhanced FW resistance (Pratap et al. 2017). Like chickpea, MAS has been deployed in groundnut breeding programs to improve several traits such as resistance to foliar diseases and nematode, and oil quality i.e. oleic to linoleic (O:L) acid ratio. For example, MABC scheme has enabled introgression of foliar disease resistant QTL from GBPD 4 (a disease resistant donor) into susceptible cultivars ICGV 91114, JL 24 and TAG 24 (Varshney et al. 2014c) and TMV 2 (Kolekar et al. 2017). In a similar manner, MAS for *ahfad2* alleles using allele specific (AS)-PCR and cleaved amplified polymorphic sequence (CAPS) markers has facilitated rapid recovery of introgression/ recombinant lines with increased oleic acid and high O:L ratio (Janila et al. 2016a, Bera et al. 2018).

### *Genomic selection and improved prediction accuracies*

Genomic selection (GS) enables identification of individuals with unobserved phenotypes exclusively based on genome wide marker data (Bohra 2013). Ability of GS to capture small effect QTL scattered throughout the genome makes this a method of choice for improving complex breeding traits. Latest development of large-scale genomic resources in legume crops has paved the way for GS implementation in crop breeding programs and initial results are encouraging (Varshney et al. 2018). For example, a set of 320 elite chickpea lines was genotyped with 3,000 DArT seq markers and the collection was evaluated for yield and related traits across two crop seasons and irrigated/rainfed conditions. High prediction accuracies for traits like 100 seed weight that are less influenced by the environment were reported by Roorkiwal et al. (2016). In another study, the same group showed how GS prediction accuracies vary based on genotyping platforms and environmental influence and found DArT seq yielding better prediction accuracies than the GBS data (Roorkiwal et al. 2018b). Also, incorporation of genotype-environment (G × E) interactions into genomic prediction model improved prediction accuracy (Roorkiwal et al. 2018b). A variety of other factors like number of DNA markers, size of training population are known to influence prediction accuracies. Though GS does not essentially require any prior information of MTAs, prediction accuracies are reported to improve following incorporation of a subset of associated loci into the GS models (Li et al. 2018).

### Sequence-based breeding in legume crops: Possibilities and challenges

Most of the traits of agronomic significance are controlled by a large number of small effect QTLs. And, first generation molecular breeding tools like MABC face great challenge while pyramiding multiple genes/QTL into single genotypes. It becomes practically difficult to genotype such large sized segregating populations that could be theoretically predicted in order to recover a genotype carrying suitable combinations of genes/QTLs. In view of the constantly decline cost of sequencing, Varshney et al. (2018) have proposed sequence-based breeding strategy for crop improvement, which entails that a larger set of founder genotypes/germplasm collection should be sequenced at greater depth followed by GWAS to identify the desirable genotypes harbouring highest number of favourable alleles and least deleterious alleles. Crossing of such superior genotypes will eventually lead to a population in which high-performing individuals could be chosen using GS models trained with founder genotypes/germplasm. The genotypes thus selected could be either directly released as a variety or recycled back into the breeding program to initiate the next round. This sequence-based strategy that seeks continuous population improvement is need of the hour in order to sustain breeding programs for delivering rapid genetic gains. In parallel, authors advocate the use of MABC/MAS for 'defect elimination' of mega varieties in the crops. For GS, population genotyping is suggested by SNP arrays or

other methods skim sequencing or GBS based on the resources available (Varshney et al. 2018).

## Conclusion and perspectives

Tremendous progress in legume genomics over the last decade has resulted in the current availability of unprecedented genomic resources. Growing application of these modern tools in crop improvement programs has witnessed a dramatic progress from training (development) phase to breeding (deployment) phase. This training-to-breeding phase progression is evident from the delivery of a variety of molecular breeding products in these crops. Many of these products are being evaluated under All India Coordinated Research Projects (AICRPs) of the respective crop in order to facilitate their possible cultivation at farmers' field in years to come. Since DNA sequencing is increasingly become affordable to researchers, the major stumbling block that hampers the crop breeding progress is accurate and precise phenotyping. However, large-scale plant phenotyping worth the investment with the adoption of new breeding methods like GS that rely on minimal phenotyping. While methods like MAS/MABC become integral part of legume breeding programs, we anticipate that breeding populations constantly improved by sequence-based breeding approach will help accelerating the genetic gains. Realization of the full potential of sequence-based breeding approach, however, will depend upon the decrease in the sequencing cost and its affordability in near future, development of cost-efficient phenotyping (field-based or automated) protocols and importantly, the ability of researchers to use analytical tools to derive meaningful inferences from the deluge of sequencing and phenotyping datasets.

## Declaration

The authors declare no conflict of interest.

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