

Molecular markers and genomic resources for disease resistance in peanut- A review

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ABSTRACT

Recent polyploidization of peanut genome and geographical isolation has rendered peanut to be a highly monomorphic species. Due to its narrow genetic base, cultivated peanut has been susceptible to various diseases, causing economic loss to farmers. Availability of only a few disease resistance sources in cultivated peanut has resulted in limited success using the conventional breeding practices. Also, scarcity of markers has been the major limiting factor to precisely identify the disease resistance genomic regions. Recent identification of large number of molecular markers using advanced genomic resources and high throughput sequencing technologies has and will continue to assist in improvement of peanut diversity and breeding. This review gives an update on recent discovery of molecular markers associated with major diseases and the available genomic resources in peanut.

Key words: Diseases, Genetic markers, Genomics, QTL.

Cultivated peanut (*Arachis hypogaea* L.) provides nutrition in the form of oil and protein worldwide and is grown on approximately 21-24 M ha of land annually (Sarkar *et al.*, 2014). It is cultivated mainly in the semi-arid tropics (SAT) regions. China, India, Nigeria and USA together produce about 69.2% of global production (FAO 2014).

Peanut genomics is thus very challenging due to its inherent complexity of genetic and genomic architecture. Due to ploidy difference between the cultivated peanut and its diploid wild relatives, the contribution of rich source of wild alleles has been insignificant in cultivated peanut improvement. Dearth of sufficient and suitable genomic resources has impeded molecular breeding activities resulted peanut to be among the less-studied crops until the availability of peanut genome.

Studies in section *Arachis* using markers like Restriction Fragment Length Polymorphism (RFLP) (Kochert *et al.*, 1991), Amplified Fragment Length Polymorphisms (AFLPs) (Milla *et al.*, 2005), Random Amplification of Polymorphic DNA (RAPDs) (Hilu and Stalker, 1995), Simple Sequence Repeats (SSRs) (Nagy *et al.*, 2012) and single nucleotide polymorphism (SNP) (Clevenger *et al.*, 2017) have demonstrated that cultivated peanut has much less diversity than the diploid species. Almost equal contribution of DNA from the two diploid progenitors (*A. duranensis* and *A. ipaensis*) makes *A.*

hypogaea a lot similar to its parents (Bertioli *et al.*, 2016). Narrow genetic base has been the major reason for disease susceptibility in peanut.

Molecular markers because of their versatility have been widely used in plant genetics and breeding activities. In order to implement marker assisted selection (MAS) in peanut, molecular markers are the key to achieve it. Recent up-thrust in genomics technologies and their application in peanut has resulted in identification of large number of SNP markers that has recently been shown to identify disease related QTLs in peanut (Agarwal *et al.*, 2018). The major diseases of peanut include tomato spotted wilt tospovirus (TSWV), early leaf spot (ELS), late leaf spot (LLS), rust, and root-knot nematode (RKN) causes economic loss to the growers all over the world. Past one decade has witnessed the progress in peanut genomics in terms of discovery of new molecular markers, generation of genetic linkage and physical maps, identification of QTLs and genes associated with major diseases. Therefore, in this review, we have focused on genomic advancements particularly in the area of disease linked markers, genetic linkage maps, and other genomic resources/applications.

Tomato spot wilt tospovirus: Tospovirus (family *Bunyaviridae*) is transmitted by thrips. TSWV disease in cultivated peanut in the southern US alone results in an average annual loss of US \$ 12.3 million. This disease can

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be controlled by applying chemical insecticide (for controlling thrips), but this will adversely affect our environment and also enhances production cost for farmers. Therefore, the permanent and environment-friendly method to solve this problem is to generate disease resistant plants by breeding and trait mapping. In order to map TSWV resistance trait in peanut an integrated map was developed using two recombinant inbred lines (RILs) derived from the crosses between Tifrunner x GT-C20 and SunOleic 97R x NC94022 designated as "T" and "S" populations respectively. This integrated map harbored a total of 324 SSRs covering genetic distance of 1352.1 cM with 21 linkage groups (LGs). The major QTL corresponding to TSWV resistance was reported on LG A01 with 35.8% phenotypic variation explained (PVE) (Qin *et al.*, 2012). A total of 38 QTLs for TSWV were identified with one common genomic region for LS and TSWV (Wang *et al.*, 2013). Similarly, a linkage map was generated from S-population with 248 marker loci. A total of 40 QTLs were identified including 6 QTLs for TSWV (PVE 4.36-29.14%) using the improved genetic maps. Out of 6 QTLs, five were identified on LG A01 and the remaining one was on A09. In this study, six major genomic regions were discovered containing QTLs controlling multiple disease resistance (Khera *et al.*, 2016).

Tseng *et al.*, (2016) studied the genetic basis of superior TSWV resistance of Florida-EPTM-113 using bulk segregation analysis. They mapped major QTLs associated with TSWV resistance in Florida-EPTM-113 by using 329 polymorphic SSR markers. Genotyping by sequencing data were also used to study association analysis, a high degree of association was found with TSWV resistance with 11 SNPs. Recently an improved genetic linkage map of T-population with 418 marker loci, with map coverage of 1935.4 cM and a marker density of 5.3cM per loci was developed (Pandey *et al.*, 2017a).

Leaf spot: The main causing agents of leaf spot in peanut are two different fungi: *Cercospora arachidicola* (ELS) and *Cercosporidium personatum* (LLS). The complexity of LLS was studied by crossing *A. duranensis* and *A. stenosperma* to generate a genetic map with microsatellite markers and legume anchor markers. This map was used to identify genomic regions controlling disease resistance including 35 candidate genes and five QTLs for LLS resistance (Leal-Bertioli *et al.*, 2009). A linkage map with a total of 268 RILs derived from TAG 24 x GPBD 4 was generated resulting in identification of QTLs for LLS (Khedikar *et al.*, 2010). Similarly, to identify LLS linked markers, a total of 188 and 181 SSR markers were used in two RIL populations named: RIL-4 (TAG 24 x GPBD 4) and RIL-5 (TG 26 x GPBD 4) to construct a consensus map with 225 marker loci onto 20 LGs. A total of 28 major QTLs (13 for RIL-4 and 15 for RIL-5) were identified in this study (Sujay *et al.*, 2012). Thirty seven QTLs were identified for LS in T-population in three different generations using

genotyping data of F₂ and F₅ generation and phenotyping data of F₈ generation with PVE 6.61-27.35% in F₂ generation and 13 QTLs with PVE 5.95-21.45% in F₅ generation (Wang *et al.*, 2013). Varshney *et al.* (2014) demonstrated that the rust resistant Introgression Lines (ILs) (developed by introgression of rust resistance QTL with PVE 82.62% from cultivar 'GPBD 4') can also show resistance to LLS. The ILs showed resistance to LLS because the targeted QTL region also has QTL for LLS resistance. An improved genetic linkage map of TAG 24 x GPBD 4 was generated by using 289 marker loci and adding 139 new SSRs and transposable element markers (AhTE) with a total map coverage of 1730.8 cM. Four QTLs were identified for LLS resistance from this study (Kolekar *et al.*, 2016). Recently an improved genetic map of S-population with 248 marker loci was generated resulting in identification of 22 QTLs for ELS and 20 QTLs for LLS (Khera *et al.*, 2016).

Recently, an improved genetic linkage map of T-population with 418 marker loci was generated (Pandey *et al.*, 2017a). A total of 9 QTLs were discovered for ELS with PVE ranging from 6.26-13.28%. For LLS, 22 QTLs including five major QTLs were discovered with PVE range of 6.40-15.55%. Further, Pandey *et al.* (2017b) analyzed the sequence data of resistant and susceptible bulk using QTL-seq approach and identified 2.98 Mb genomic region for LLS resistance.

Rust: Rust in peanut is caused by *Puccinia arachidis* Sp. Rust not only adversely affects the yield and pod quality, but also affects the yield and quality of haulm. The most convenient and eco-friendly solution of this problem is host-controlled disease resistance. Genetic mapping and QTL analysis for rust disease was done on RIL mapping population derived from TAG 24 x GPBD 4. The QTL analysis for total six stages and five different environments resulted in identification of 12 putative QTLs for rust on eight different LGs (Khedikar *et al.*, 2010). Further, Sujay *et al.* (2012) generated a genetic map with a total of 206 and 205 polymorphic SSRs in two RIL populations, RIL-4 and RIL-5 respectively. A total of nine QTLs with PVE 2.54-82.96% in RIL-4 and six QTLs with PVE 2.89-78.96% in RIL-5 were identified. In another study, a RIL population of 164 individual derived from resistant (VG9514) and susceptible (TAG 24) parents were used for screening of rust resistance in five environments by using 109 SSR markers (Mondal *et al.*, 2012a). Similarly, Mondal *et al.* (2012b) discovered 259 EST-SSR markers from 5,184 *A. hypogaea* ESTs. These EST-SSR markers along with 34 resistance genes candidate markers were used for association and genetic mapping in cultivated peanut. Marker-assisted backcrossing (MABC) was used for introgressing the QTL region controlling rust resistance with PVE up to 82.62% from the resistant cultivar 'GPBD 4' into three rust susceptible peanut varieties named 'ICGV 91114', 'JL 24' and 'TAG 24'. Similarly, using MAS in generating disease resistant crops, Leal-Bertioli *et al.*

(2015) identified genomic regions controlling different components of rust resistance in RILs generated by crossing *A. ipaensis* (rust susceptible) and *A. magna* (rust resistant). A total of 13 QTLs were identified. A major QTL was identified on B08 for rust resistance with PVE range of 2.8-59.3%. Kolekar *et al.* (2016) identified five QTLs for LLS and rust using TAG 24 x GPBD 4 RILs. Three out of five QTLs showed high PVE for both LLS and rust and the other two showed high PVE only for rust. Further, Pandey *et al.* (2017b) identified 3.06 Mb genomic region on chromosome A03 for rust resistance using QTL-seq approach by analyzing the resistant and susceptible bulks.

Nematode: Root-knot nematode (RKN) (*Meloidogyne* sp.) is economically important plant parasite with a very wide host range. In case of peanut three RKN species are identified as pathogens, namely, *M. arenaria* (Neal) Chitwood, *M. hapla* Chitwood, and *M. javanica* (Treub) Chitwood. Among these three, *M. arenaria* is mainly responsible for reduction in peanut product in infested field. Cultivated peanut possess very low level of resistance against RKN. However, wild diploid species of peanut is important for identification and introgression of resistance genes into cultivated peanut. *A. glabrata* was identified for the first time to have high level of resistance against *M. arenaria* (Baltensperger *et al.*, 1986). Similarly, two wild diploid species, *A. batizocoi* and *A. cardenasii* showing genetic compatibility with *A. hypogaea* were found to have strong resistance against *M. arenaria* (Nelson *et al.*, 1990). In order to identify more RKN resistant sources from *Arachis* sp., two peanut germplasm lines TxAG-6 and TxAG-7 were identified as *M. arenaria* resistant (Simpson *et al.*, 1993). Furthermore, TxAG-7 was used as donor parent to generate BC₄F₂ population for identification of RAPD markers associated with RKN resistance (Burow 1996). Garcia *et al.* (1996) identified first RAPD, sequence characterized amplified region (SCAR) and RFLP markers closely associated with two dominant genes responsible for reduction in egg number (*Mae*) and galling (*Mag*). Similarly, Choi *et al.* (1999) studied the genetics and mechanism of resistance to *M. arenaria* in six BC₅F₂ peanut breeding lines (*A. hypogaea* × TxAG-7 with Florunner as recurrent parent). They discovered three RFLP probes; two out of three probes (R2545 and R2430E) showed their proximity to the resistance allele. Further, two RFLP loci associated with a RKN resistant gene was utilized for identification of homozygous individual for RKN resistance (Church *et al.*, 2000). Furthermore, elevated level of resistance against *M. arenaria* was observed in a runner market type peanut (2n=4X=40) named “COAN”. This was the first RKN resistance peanut cultivar. COAN was generated by utilizing backcross introgression pathway (Simpson, 1991). COAN was developed by crossing *A. cardenasii* and *A. diogoi*, the resultant F₁ hybrids was further crossed with *A. batizocoi*. The chromosome of the resulting hybrids (2n=20) was doubled by using colchicine to form TxAG-6. This was

further crossed with Florunner and after five backcrosses, the desired line with RKN resistance, TP262-3-5 (COAN) was identified (Simpson and Starr 2001). Similarly, a backcross population was obtained by crossing *A. batizocoi* with *A. cardenasii* × *A. diogoi*. This population was used for introgression of chromatin from donor diploid species into the genome of cultivated peanut. This population was also used for the development of first molecular map of tetraploid peanut. A total of 370 RFLP loci were mapped on 23 LGs with a total coverage of 2210 cM (Burow *et al.*, 2001). GP-NC WS 5 and GP-NC WS 6 were registered as RKN resistant peanut germplasm lines. The two resistant lines were obtained from an interspecific crossing between *A. hypogaea* and *A. cardenasii* (Stalker *et al.*, 2002). Another cultivar, “NemaTAM” was registered as RKN resistant cultivar. NemaTAM was generated from an introgression pathway (Simpson, 1991) and Florunner was used as the recurrent parent in backcross (Simpson *et al.*, 2003). In search of more nematode resistant peanut cultivars, “Tifguard” was developed. This was the first cultivar with resistance against both RKN and TSWV. Tifguard was developed by hybridizing TSWV-resistant ‘C-99R’ with COAN (nematode resistant) (Holbrook *et al.*, 2008). Tifguard (RKN resistant) was utilized as recurrent female parent to generate a cultivar with nematode resistance, and Georgia-02C, Florida-07 were used as contributors for high oleic acid/linoleic acid (O/L) ratio trait. The resultant cultivar “Tifguard high O/L” showed nematode resistance and high O/L ratio (Chu *et al.*, 2011). Similarly, a nematode resistant, high yielding and with high O/L ratio cultivar named “Webb” was released (Simpson *et al.*, 2013). Further, Leal-Bertioli *et al.* (2016) crossed *A. duranensis* and *A. stenosperma* to study RKN resistance in wild *Arachis* sp. *A. stenosperma*. Results showed QTLs present on LG 02, 04, 09 to be strongly associated with nematode root galling and egg production.

Diversity-based genomic resources for peanut and future prospects

Prudent use of available genetic resources is the major avenue for further improvement of peanut crop (Fig.1). ICRIASAT headquarters based in India has the largest collection of peanut germplasm of over 15,446 accessions from 92 countries (Nugrahaeni and Rahayu, 2017) followed by NBPGR, India with 14,585 accessions and the DGR, ICAR, India with 9,024 accessions (see Pandey *et al.*, 2012). These accessions also include sources for major biotic stresses like ELS, LLS, rust etc. Similarly, OCRI, China is also maintaining germplasm collection with 6,839 accessions (Jiang *et al.*, 2012) and USDA, USA with 9,917 accessions. Along with gene bank, ‘core collection’ of peanut is also available in many countries. The core collection is 10% of the total germplasm collection and generated by selecting the suitable lines according to the specification of breeding experiment. There is a U.S. core collection of 831 accessions (Holbrook *et al.*, 1993) followed by 1704 genotype

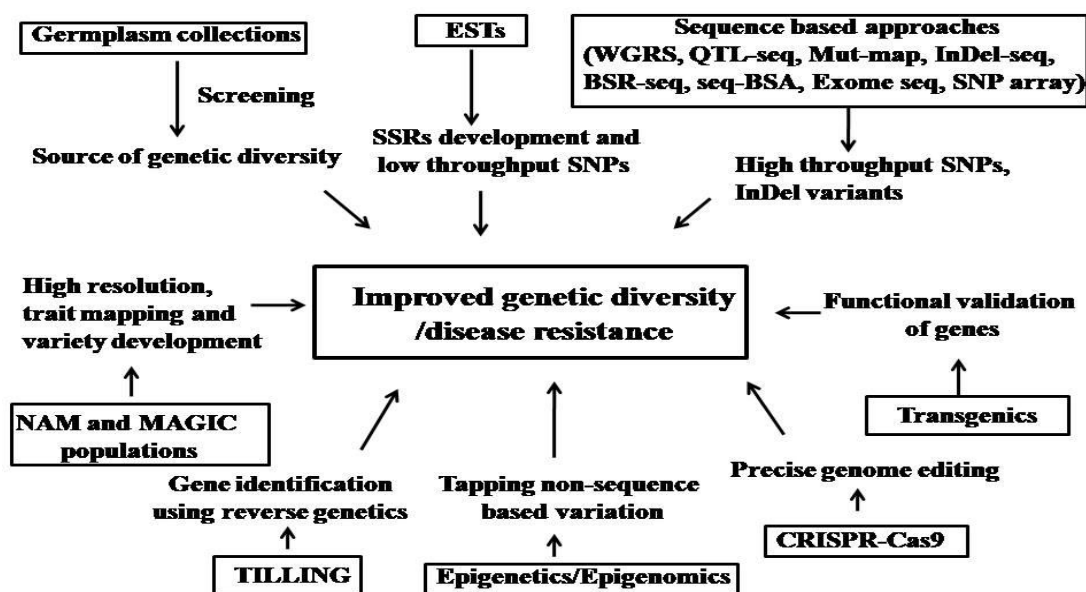


Fig 1: Outline showing genetic and genomic resources, and genomic applications to increase genetic diversity and resistance to pathogens in peanut.

accessions of *A. hypogaea* at ICRISAT, India (Upadhyaya *et al.*, 2003) and the Chinese core collection with 576 accessions (Jiang *et al.*, 2008). Similar to core collection, 'mini-core collection' is also developed with even lesser number of genotypes (10% of core collection or 1% of entire germplasm collection). There is a mini-core collection developed at ICRISAT, India with 184 accessions (Upadhyaya *et al.*, 2002). US and China have 112 and 298 accessions respectively (Holbrook and Dong 2005, Jiang *et al.*, 2010) as mini-core collections. Center for Biotechnology and Genetic Resources (BB-Biogen) has a peanut germplasm collection with 1,194 accessions (Sumarno, 2007). These genetic resources are the source of genetic diversity and genetic variation and are being utilized in peanut crop improvement. Similarly, 554 accessions of peanut are conserved in Indonesian Legumes and Tuber Crops Research Institute (Iletri) (Nugrahaeni and Rahayu 2017).

Until 2015, when the whole genome sequence of diploid progenitors of peanut were not available, transcriptome data and EST were used to study the gene expression and identification of candidate genes. Bosamia *et al.* (2015) recognized 2,784 SSR containing sequence and studied association of these SSRs with biotic and abiotic stresses. Currently, there are a total of 281,763 ESTs available for *Arachis* sp. on NCBI (www.ncbi.nlm.nih.gov accessed October 18, 2017). Out of these, *A. hypogaea* contributed 205,442 ESTs, 745 ESTs from *A. hypogaea* subsp *fastigiata*, diploid progenitors of cultivated peanut *A. duranensis* have 35,291 and *A. ipaensis* have 32,787 ESTs. The number of ESTs in other peanut sp. is as following: *A. stenosperma*

(6264), *A. magna* (750), *A. diogeni* (75) and *A. appressipila* (400). Many of these ESTs are associated with various biotic stresses like 8000 ESTs were identified in *A. stenosperma* roots infected by *M. arenaria* (Proite *et al.*, 2007), 21,777 ESTs were identified in seeds against *Aspergillus* infection followed by aflatoxin contamination (Guo *et al.*, 2008), 16,931 ESTs were discovered in leaves tissues infected with TSWV and LS disease (Guo *et al.*, 2009).

Recent availability of genomes of diploid progenitors of tetraploid peanut has opened avenues for marker discovery and analysis of genetic diversity in peanut (Bertioli *et al.*, 2016; Chen *et al.*, 2016). Whole genome re-sequencing (WGRS) of populations ("T" and "S") and their parents (Tifrunner x GT-C20 and Sunoleic 97R x NC94022) have resulted in identification of several thousands of SNP markers that are being deployed in construction of very high-density linkage maps and fine map the QTLs for resistance against disease like ELS, LLS and TSWV (Agarwal *et al.*, 2018). Further, to enhance the genetic resources and demonstrate its ability to perform diversity studies in peanut, a 58K SNP array has been developed (Pandey *et al.*, 2017c, Clevenger *et al.*, 2017). Efforts are also in progress to come up with the tetraploid (Tifrunner) genome assembly and this will be a tremendous boost for genomics assisted breeding in peanut. Currently, the use of synthetic tetraploid assembly (diploid A- and B-genomes together) has undoubtedly helped the breeding community to have robust markers, however, the genetic rearrangements of the actual tetraploid remain obscure in this scenario. Thus, the availability of tetraploid genome assembly will further strengthen the breeding practices at a much rapid rate with more confidence.

Genetically engineered (transgenic) peanut either through overexpression or RNAi mediated suppression of genes coding for cell wall component, pathogenesis-related proteins, oxalate oxidase etc. has resulted in resistance to various fungal, virus and insect pest (Krishna *et al.*, 2015). However, with no transgenically developed cultivar released commercially so far (Mishra *et al.*, 2015), and the issues associated with commercialisation of transgenic crops, it is imperative to focus on genomics assisted breeding approaches to break the barrier of low genetic diversity in peanut.

QTL-seq has been another approach of choice when it comes to crops with large genome size. In a recent study, it has successfully been implemented to dissect rust and LLS disease resistance traits in peanut (Pandey *et al.*, 2017b). Other high throughput NGS based approaches like Mutmap (Abe *et al.*, 2012), Indelseq, and bulked segregant RNA-seq (BSR-seq) (Liu *et al.*, 2012) can be explored in case of peanut to target the regions of interest in the genome.

Nested association mapping (NAM) combines the positives of linkage mapping and association mapping and has been an effective multi-parental mapping population strategy to map the important genomic regions with high-resolution. Power of NAM has been demonstrated in maize (Yu *et al.*, 2008) and other crops. In peanut, two structured mapping populations have been developed using two x eight (common by unique) factorial nested association mapping design, each with four founders and a common parent. A total of eight recombinant inbred line (RIL) populations (2 x 4) with more than thousand RILs have been developed that maximize genetic diversity for practical breeding objectives. The two common parents for NAM include Tifrunner and Florida-07, and the eight unique lines include N08082oIJCT, C76-16, NC 3033, SPT 06-06 (Holbrook *et al.*, 2013). The RILs derived from common and unique parental crosses segregate for ELS, LLS, TSWV resistance, preharvest aflatoxin contamination, drought tolerance and pod fill.

MAGIC (multiparent advanced generation intercross) population are permanent mapping populations for precise QTL mapping and variety development. One of the main benefits of the MAGIC populations is the creation of new combinations of alleles through generations of mixing founder genomes together (Huang *et al.*, 2015). Three MAGIC populations are under development targeting different trait combinations. Targeting traits like fresh seed dormancy, oil content, seed mass, kernel Fe and Zn content, aflatoxin tolerance, stem rot tolerance, and PBND tolerance, the first MAGIC population (ICGV 88145, ICGV 00308, ICGV 91114, ICGV 06040, ICGV 00440, ICGV 05155, GPBD 4, and 55-437) was developed. The second MAGIC population was developed with aspergillus resistance and

aflatoxin contamination as targets, while the third one focussed on components of drought tolerance (see Pandey *et al.*, 2016).

Targeting induced local lesions in genome (TILLING) is an important reverse genetics approach to identify the causal genes in the genomes. Knoll *et al.* (2011) used Ethyl methane sulfonate (EMS) to generate mutations in peanut. Homeolog pairs of a major allergen gene (*Ara h1/1.02* and *Ara h 2.01/2.02*) and fatty acid desaturase, *Ah FAD2A/B* (a major gene controlling the ratio of oleic/ linoleic acid in peanut seeds) were screened in the mutagenized population to discover functional mutations in these genes. Currently, F₃ generation populations are being grown and phenotyped in the fields (Tifton, GA) for LS and prominent main stem feature. A total of 25 lines comprising of 16 susceptible and 9 resistant lines for LS, and 11 lines with presence and 14 lines with absence of the prominent main stem from the population has been sequenced. Also, two mapping populations from these TILLING lines namely T47-7 (resistant to leaf spots) x T33-3 (susceptible to leaf spots) and T90-1 (presence of stem) x T71-2 (absence of stem) are in progress.

Epigenetic studies have so far been the least explored area in case of peanut. Epigenetic changes contribute to plant phenotypic flexibility of their reversible nature. Epigenetics can provide the basis of non-sequence based variations in peanut. Epigenetic changes in the genomes can be explored in terms of DNA methylations or histone modifications. For example in developing peanut embryos epigenetic investigation of peanut allergen gene, *Arah3* showed an association between the H3 histone modification and increased expression of *Arah3* during embryo maturation (Fu *et al.*, 2010). Epigenetic assisted breeding using epigenetic recombinant inbred lines (epiRILs) has also been shown to validate the theory that epigenetics contributes substantially to variation in plant growth, morphology, and plasticity, especially under stress conditions (Kooke *et al.*, 2015). Such studies will be very interesting to find answers to the problems in peanut that are not explained by Mendelian genetics.

CONCLUSION

Modern breeding practices supported by genomics provides promising platforms like SNP based genome wide association mapping (GWAS), NAM, MAGIC and large Hap Map data sets can identify the causal allele that can be transferred into modern cultivars. Availability of tetraploid reference genome in near future will take cultivated peanut breeding to the next level as the reference assembly will aid in generating plethora of robust markers that will be implemented in marker assisted breeding. With an overall objective of broadening the genetic base of an important crop like peanut efforts are needed to introduce novel genetic

variations using the above mentioned techniques that can at the same time also lead to crop improvement.

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