Classical and Molecular Approaches for Mapping of Genes and Quantitative Trait Loci in Peanut

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Abstract

Advances in availability of genomic resources coupled with genetic resources have accelerated the process of developing better understanding of cytogenetics and genetics of peanut using modern technologies. The cytogenetic studies provided greater insights on chromosomal structures and behaviour of different *Arachis* species along with their genetic relationship with each other. Researchers are moving faster now in using single nucleotide polymorphism (SNP) markers in their genetic studies as simple sequence repeats (SSRs) did not provide optimum genome density for genetic mapping studies in peanut. Due to availability of reference genome of diploid progenitors, resequencing of some genotypes and soon to be available tetraploid genome, a high-density genotyping array with 58 K SNPs is now available for conducting high-resolution mapping in peanut. ICRISAT has developed next generation genetic mapping populations such as multi-parent advanced generation intercross (MAGIC) and nested association mapping (NAM) populations for conducting high-resolution trait mapping for multiple traits in one go. Affordability of sequencing also encouraged initiation of sequence-based trait mapping such as QTL-seq for dissecting foliar disease resistance trait. Few successful examples are available in peanut regarding development of diagnostic markers and their deployment in breeding to develop improved genotypes, which may see a significant increase in coming years for developing appropriate genomics tools for breeding in peanut.

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7.1 Introduction

Cultivated peanut (*Arachis hypogaea* L.), also called as groundnut, is the second largest oilseed legume crop after soybean in the world. It covers the tropical and subtropical regions but primarily grown in the semi-arid tropics (SAT) regions of the world. This crop is cultivated in >100 countries of Africa, Asia and Americas and is consumed in almost all the countries in one or the other form. The global annual production in 2014 was 42.31 metric tons from an area of 25.44 million hectares (FAO 2014, accessed on 10th March 2016).

Peanut productivity is highly affected by several biotic and abiotic stresses across the world. The major abiotic stresses include terminal drought, heat and salinity (Fig. 7.1). The major biotic stresses include rust, early leaf spot (ELS) and late leaf spot (LLS), tomato spotted wilt virus (TSWV), groundnut rosette disease (GRD), peanut clump virus disease (PCVD), peanut stripe virus (PStV), peanut bud necrosis disease (PBND), peanut stem necrosis disease (PSND), bacterial wilt and root-knot nematodes (Nigam et al. 2012). The above-mentioned stresses cause massive yield loss in different intensities and quantity in addition to deteriorating the quality of the produce. In addition to above yield reducing stresses, *Aspergillus flavus* infection is a very serious issue as it produces carcinogenic mycotoxins known as aflatoxins which have an adverse impact on human health and the economy. Aflatoxin is known to cause cancer in human beings, animals and poultry birds that are fed with contaminated peanut seeds/cakes. The major causal agent for aflatoxin contamination, *A. flavus,* is predominant species in Asia and Africa while *A. parasiticus* in the USA (see Pandey et al. 2012a; Janila and Nigam 2013). Further, recent increased awareness among consumers has also raised demand for good oil quality and nutritious peanut seed.

The domesticated peanut is an allotetraploid ($2n = 4x = 40$) crop with two subgenomes

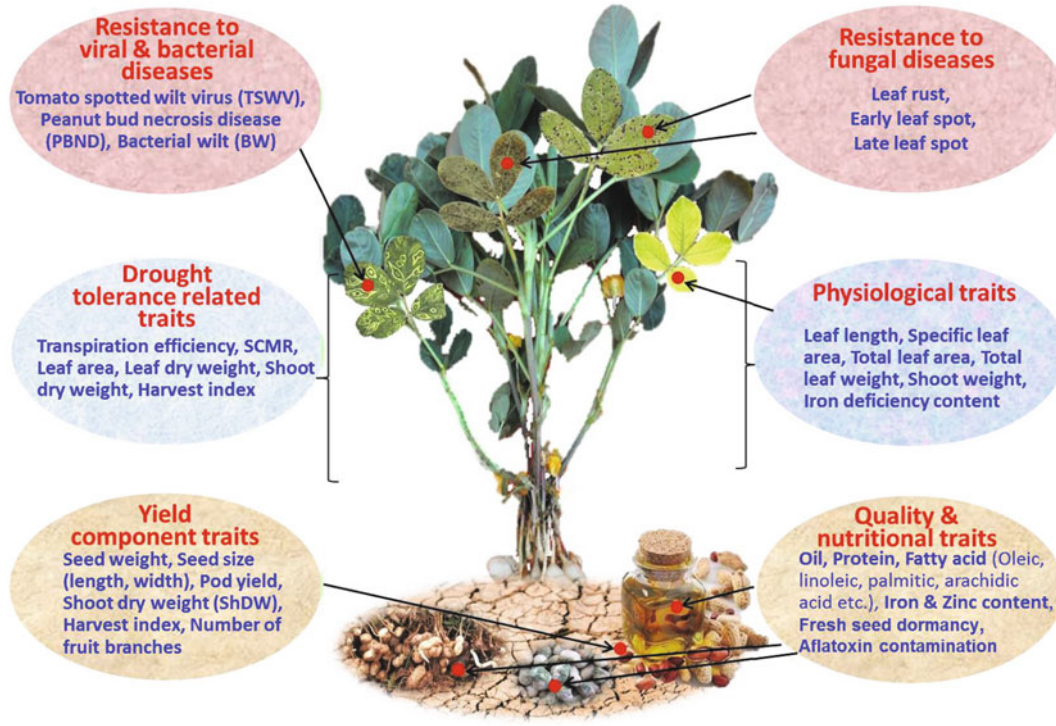

Fig. 7.1 Trait mapping efforts in peanut for developing trait-linked markers

(A and B). About 3500 years ago, these two subgenomes believed to have come together from a single hybridization event between two diploid wild species ($2n = 2x = 20$) accompanied by whole genome duplication. The above event gave rise to cultivated peanut which then remained isolated over the centuries from its progenitors' genepool for further diversification. As a consequence, the limited evolutionary history, coupled with hybridization barriers between diploids and the tetraploid, have created roadblocks in the mobilizing alleles from wild species to the cultivated peanuts leading to narrow genetic base for today's cultivated peanut (Simpson 1991). Amalgamation of the genomics with the integrated classical breeding has calibre to boost the yield of peanut by overcoming selected genetic barriers. Since a decennary, enormous progress has been made in the peanut genomics leading to the development of enormous genetic and genomic resources such as genome sequences, whole genome re-sequencing (WGRS), molecular markers, mapping populations, genetic maps, high throughput sequencing and genotyping platforms, transcriptome sequencing and proteome (Pandey et al. 2012a, 2016a; Varshney et al. 2013, 2015a, b). These resources have been exploited and utilized in genetic map construction, quantitative trait loci (QTL) mapping for traits, association mapping and ultimately transform it in the translational genomics for the improvement of peanut (Pandey et al. 2014a, 2016a; Varshney et al. 2015b).

Genetics and genomics offer excellent opportunity to accelerate genetic gains and achieve developing improved peanut varieties with high yield and quality. In the case of peanut, such technologies will contribute to improving the biotic and abiotic resistance, oil quality, seed quality, seed nutrition and yield. For increasing genetic gains in breeding programs, efficient utilization of genetic resources conserved in available germplasm through genomics approaches is essential. Development of superior varieties with the improved characteristics keeping in mind the requirements of a specific environment, growers and consumers will eventually enhance the chances of adoption, which unfortunately touches quiet low now. Genomics utilizes the analysis of full genetic constitute by tagging, sequencing and functional examination to discover genes/QTLs that operate, check and alter the expression. Plant breeding along with genetics and genomics is a potent way to give phenomenal growth to agriculture productivity and sustainability. Advances in next-generation sequencing (NGS) technologies has accelerated the pace in crop genetics and breeding (Varshney et al. 2009a). Peanut Genome Consortium (PGC) with the collaboration of international partners initiated the International Peanut Genome Initiative (IPGI) in 2012 and released the first chromosomal-scale draft sequences of two progenitors of tetraploid cultivated peanut (*A. hypogaea*), representing A-genome (*Arachis duranensis,* accession V14167) and B-genome (*A. ipaensis,* accession K30076) (Bertioli et al. 2016). In a parallel effort by Diploid Progenitor A-genome Sequencing Consortium (DPPAGSC) (http://ceg.icrisat.org/dppga/Manuscript.html), another draft sequence of A-genome progenitor (*A. duranensis*, accession PI475845) has also been developed and made available in the public domain (Chen et al. 2016a). The IPGI-led genome assembly of A-genome progenitor is better than the DPPAGSC-led genome assembly in terms of quality and applicability in further peanut genomics research. Nevertheless, the DPPAGSC-led genome assembly provided in-depth genome analysis identifying genes for geocarpy, oil biosynthesis and allergens. The above-mentioned genome assemblies will further enhance the genomics research leading to gene discovery, high-resolution trait mapping and molecular breeding.

This chapter provides updates on cytological studies, molecular markers, genetic linkage maps and trait linked QTL identification using linkage and association mapping/linkage disequilibrium mapping approaches. Also, we discuss the development of complex high-resolution trait mapping populations like MAGIC (multi-parent advanced generation intercross) and NAM (nested association mapping). In addition, we

discussed NGS-based SNPs identification linked to gene/QTLs for concerned traits using modern high-resolution trait mapping and gene discovery approaches.

7.2 Advances in Peanut Cytological Research

With the generation of huge data through NGS technologies, the challenge comes in computational analysis. The advanced plant cytogenetics has made essential contributions to genomics by interpreting the scaffolds, marker orders, genome arrangements like translocations and inversions. Chromosome markers developed using fluorescent in situ hybridization (FISH) with rDNA probes and fluorescent banding were used in development of chromosome map of peanut (Robledo and Seijo 2010). FISH is commonly used to map unique or low copy number sequences and to localize repetitive sequence to produce chromosome recognition cocktails or explore genome relations in polyploid or closely related plant species. Chromosome identification in peanuts started with studies carried out by Husted (1933), who delineated the occurrence of two pairs of chromosomes in peanut. Karyotyping analysis and relationships among varieties of *A. hypogaea* L. were studied (Stalker and Dalmacio 1986) and later, the relationship of *Arachis* section was cytologically implicated (Stalker et al. 1991). Development of fluorescent banding patterns (like Q-, C-, G-, R-, T-banding) revolutionized the karyotyping and characterization of the genomes of different plant species. The fluorochrome banding patterns acted like markers to differentiate different species of *Arachis* section (Raina and Mukai 1999; Seijo et al. 2004). Modified genomic in situ hybridization (GISH) techniques were used to study the genomic relationships between the cultivated peanut and its probable progenitors (Seijo et al. 2007). Lately, sequential GISH-FISH method was utilized to study the chromosome analysis of peanut (Pei et al. 2015).

Cytogenetic studies have been very important to distinguish and define different genomes of *Arachis* section including the first genome constitution establishment within the *Arachis* genus. Based on thorough cytological studies, the *Arachis* species were categorized to have A genome and non-A (B, D, E, F, K, P) genomes (Smartt et al. 1978; Smartt and Stalker 1982; Stalker 1991; Robledo and Seijo 2010). Interestingly, the species within each subgroup were more closely distributed geographically and were named using geographical reference (Robledo et al. 2009). The karyotype features of *A. duranensis* and *A. cardenasii* indicated the occurrence of 'A' genome; *A. ipaensis* of 'B' genome; *A. glandulifera* of 'D' genome; *A. batizocoi*, *A. cruziana*, *A. krapovickasii* of 'K' genome; and *A. benensis*, *A. trinitensis* of 'F' genome. The origin and evolution of peanuts have been studied based on the rDNA, internal transcribed spacer (ITS) region, restriction fragment length polymorphism (RFLP) markers. *A. duranensis* and *A. ipaensis* were proposed to be the probable progenitors of cultivated peanut using RFLP analysis (Burow et al. 2009). Later based on the studies of rDNA using FISH and heterochromatin distribution showed that the A genome of the cultivated peanut was more related to *A. duranensis* and B genome was related to *A. ipaensis* (Seijo et al. 2004; Robledo and Seijo 2010).

With advances in technologies and modification of existing technologies like spectral karyotyping (SKY) and DNA fibre-FISH can be used in more accurate physical mapping. During SKY, all the chromosomes can be simultaneously visualized using chromosome specific probes (Imataka and Arisaka 2012). In DNA fibre-FISH technique, the extended DNA fibres released from lyzed nuclei are used as specimens for hybridization instead of nuclei or chromosomes as for conventional FISH. Fibre FISH can distinguish two probes separated by 1 kb on a DNA fibre (Wang et al. 2013). Although there are no reports yet in peanut, there is high scope to use these new techniques in characterizing the wild relatives. These crop wild relatives (CWR) have untapped genomic regions that confer resistance

to biotic stresses like ELS, LLS, PBND, PStV, PMV, TSWV, aflatoxin, corn ear worm, southern corn root worm, thrips, leaf hoppers and Spodoptera (Dwivedi and Johri 2003, 2008; Upadhyaya et al. 2011).

QTLs for disease resistance were reported from crosses involving wild diploid species in peanut (Bertioli et al. 2009) and the derivatives from the wild (Gowda et al. 2002). Besides, introgression of disease resistance genes has also been reported from the wild diploid species (*A. cardenasii*) into an elite peanut variety (Simpson and Starr 2003; Simpson et al. 2003). Very recently, introgression of rust resistance region into three elite cultivars was reported, where the donor GPBD 4 is the second cycle derivative of interspecific hybridization (Varshney et al. 2014). The introgression lines can be used for breeding and mapping of genes/QTLs simultaneously (Alpert and Tanksley 1996; Tanksley et al. 1996; Tanksley and McCouch 1997) through advanced backcross-QTL (AB-QTL) approach (Iyer-Pascuzzi et al. 2007). Synthetics developed from wild species were used to develop chromosome segment substitution line (CSSL) and used to dissect plant morphology in case of peanut (Fonceka et al. 2012). Further, CSSL intercosses and CS-RILs can be used to dissect the complex traits involved in resistance to biotic and abiotic stresses.

7.3 Genetic Markers

DNA markers have played very crucial role in forming backbone of genomics, with the utilization in genetic mapping, genomic assisted breeding (GAB), association studies, genomic selection and fine mapping (Hyten et al. 2010). During the 90s, apart from DNA marker, isozyme a protein-based marker system was deployed for the genetic diversity in peanut (Lacks and Stalker 1993). Shortly, DNA-based marker systems viz. RFLP (Kochert et al. 1996), random amplified polymorphic DNA (RAPD) (Subramanian et al. 2000) and amplified fragment length polymorphism (AFLP) (He and Prakash 1997; Herselman 2003) replaced isozyme completely. Meanwhile, several other DNA-based markers also reported like sequence-related amplified polymorphism (SRAP) (Wang et al. 2010), single strand conformational polymorphism (SSCP) (Nagy et al. 2010), and miniature inverted-repeat transposable elements (MITEs) (Shirasawa et al. 2012a). Notwithstanding, they were rarely utilized for peanut genotyping. With the most promising and reliable technology, attention of peanut researchers shifted towards development of simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) which rely on sequence information.

SSR markers are regarded as the marker of choice, because of several worthy properties viz. co-dominance, reproducibility, high variability, broad genome coverage and easy to use (Gupta and Varshney 2000). Development of SSR markers in *Arachis* came into existence in the year 1999, although in very less number, i.e. 26 SSRs yet an important initiative in peanut genomics studies (Hopkins et al. 1999). Nevertheless, >15,000 genomic as well as genic SSRs have been developed in peanut in last 15 years (Guo et al. 2013; Shirasawa et al. 2012a; Pandey et al. 2016a). Several of these markers are still not available to the global peanut research community. Few studies were also carried out to check usefulness of these markers by checking polymorphism in different germplasm sets including parents of mapping population, construction of genetic maps, marker-trait association analysis and also molecular breeding (Pandey et al. 2012b; Varshney et al. 2013).

A total of 199 highly informative SSRs with >0.50 PIC were reported after screening 4485 SSR markers (Pandey et al. 2012b). This study also reported >900 novel SSR markers, which were made accessible to the global peanut research community. Similarly, Zhao et al. (2012) and Macedo et al. (2012) reported 1343 and 78 polymorphic SSRs after screening 9274 and 146 SSRs, respectively. Recently, by using EST database available in public domain, Peng et al. (2016) reported development of 6455 SSR markers, of which only 339 SSRs amplified and only 22 were found polymorphic. Thanks to the genome

sequencing effort which has now ended the scarcity of genetic markers in peanut and now lakhs of SSRs are available due availability of draft genome sequences of the ancestor genomes (Bertioli et al. 2016; Chen et al. 2016a). Two research groups exploited the reference genome of diploid progenitors and made available primers for >150K SSRs (Luo et al. 2017; Zhao et al. 2017).

SNPs acts as a direct marker because a nucleotide base is the smallest unit of inheritance, the sequence information provides the accurate nature of the allelic variation. This sequence variation affects the development of the organism and their response to the environment. Ample amount of SNPs are dispersed in the genome, one SNPs at each 100–300 bp (Gupta et al. 2001). SNP markers are invaluable as a tool for genome wide association analysis and genomic selection offering the potential for generating ultra-high-density genetic maps. SNP development is difficult in peanut due to allotetraploidy that provides the abundance of polymorphism amongst homoeologous genomes, i.e. A and B genome (Dwivedi et al. 2007). Accordingly, development of SNP is very low in peanut. A 1536 SNPs Illumina GoldenGate array were designed by comparing sequences of 17 tetraploid genotypes with Tifrunner's transcriptome in the University of Georgia (Nagy et al. 2012).

In order to deploy SNPs in breeding program, kompetitive allele-specific polymerase chain reaction (KASP) assay markers appear as a good choice and cost effective. In this context, KASP assay for 90 SNPs were developed and deployed for genetic diversity in a very diverse germplasm panel, i.e. 'Reference Set' (Khera et al. 2013). Likewise, at the University of California-Davis, 768-SNP Illumina GoldenGate array was developed (see Varshney et al. 2013). Also, robustness of KASP assays was validated for SNPs in peanut (Chopra et al. 2015). A high-resolution melting, also used for an alternative SNP assay to validate SNPs in peanut (Hong et al. 2015). Nonetheless, Genotyping-by-sequencing (GBS) is another approach based on NGS technology which led one step forward to mine SNP markers for use in genetic analyses and genotyping (Beissinger et al. 2013). It is a low cost technology where there is less sample handling, PCR and purification steps and multiplexed based on precise barcoding (Davey et al. 2011). In peanut, GBS has been well demonstrated where the SNP markers developed were used to construct linkage map and QTL analysis in cultivated peanut (Zhou et al. 2014; Zhao et al. 2016). Completion of genome sequencing in 2016 for both the diploid progenitors will now facilitate SNP discovery in large scale. Recently, ICRISAT together with University of Georgia has developed SNP array with 58,000 informative SNPs (Pandey et al. 2017a). Development of such array will now facilitate generation of high-density genotyping data and high-resolution genetic mapping for trait discovery and diagnostic marker development for trait of interest. Such high throughput genotyping system will also facilitate deployment of modern breeding approaches in peanut wherein genome-wide SNP-based genotyping is essential for achieving higher genetic gains with more precision.

7.4 Genetic Linkage Maps for Diploid and Tetraploid Peanuts

Identification of molecular markers leads to construction of genetic maps and detection of genes/QTLs. Since last decennary, numbers of mapping populations and linkage maps have been developed for diploids (A and B sub-genome) and tetraploid (AABB-genome) peanut (Pandey et al. 2012a). Notwithstanding, these genetic maps were not up to mark as they had less number of markers and low density. Now, NGS-based techniques are available to identifying SNPs to enrich these maps with more number of markers and density (Table 7.1).

7.4.1 Diploid Genetic Maps

Genetic map construction was first initiated for the diploid (AA) genome leading to development of five separate maps using F_2 population by deploying a range of markers such as RFLP, AFLP and RAPD, and later SNP markers in peanut

Table 7.1 List of genetic maps constructed in the *Arachis* species using different molecular markers

S. N.	Genome	Population used	Marker loci mapped	Marker type	Linkage groups	Total map distance (cM)	Inter-marker distance	References
1	AA	F2	102–1724	AFLP, RFLP, SNP, SSR, SCAR, SSCP	10–12	1063–1230.89	3.88–10.47	Halward et al. (1993), Milla (2003), Moretzsohn et al. (2005), Nagy et al. (2012)
		BC1F1	384–437	RAPD, RFLP	11	800	3.88	Garcia et al. (2005)
		F5	170–1724	AFLP, RFLP, SSR, SCAR, SNP, SSCP, transposon	10–11	544–705.10	1.24–1.84	Shirasawa et al. (2013), Bertioli et al. (2014)
2	BB	F2	149–449	SSR	10–16	1278.6–1294	2.84–8.68	Moretzsohn et al. (2009), Guo et al. (2012)
		F6	680	SSR, transposon	10	461	0.68	Shirasawa et al. (2013)
3	AABB	F2	12–1452	AFLP, SRAP, SSR, DArT, DArTseq, transposon	5–22	139.40–3525.8	1.7–11.61	Herselman et al. (2004), Wang et al. (2012, 2013), Shirasawa et al. (2012a, b), Vishwakarma et al. (2016), Chen et al. (2016b), Shasidhar et al. (2017)
		BC1F1	298 and 370	RFLP, SSR	21–23	1843.7–2210.0	5.97–6.18	Burow et al. (2001), Foncéka et al. (2009)
		RILs	29–1685	SSR, CAPS, SNP	8–26	401.7–2208.2	0.85–18.55	Jiang et al. (2007), Hong et al. (2008, 2009), Varshney et al. (2009b), Peng et al. (2010), Ravi et al. (2011), Sarvamangala et al. (2011), Hong et al. (2010), Khedikar et al. (2010), Sujay et al. (2012), Gautami et al. (2012a, b), Qin et al. (2012), Mondal et al. (2012), Zhou et al. (2014), Mondal et al. (2014a, b)

(continued)

Table 7.1 (continued)

S. N.	Genome	Population used	Marker loci mapped	Marker type	Linkage groups	Total map distance (cM)	Inter-marker distance	References
		F6	772–1261	SNP, SSR and transposon	20	1442–1487.3	1.14–1.92	Shirasawa et al. (2013), Bertioli et al. (2014)
		F8	237	SNP, SSR	20	1627.4	6.8	Zhao et al. (2016)
4	**Integrated genetic maps**							
	AABB	3 RILs	175	–	22	885.4	5.06	Hong et al. (2010)
	AABB	2 RILs	225	–	20	1152.9	5.12	Sujay et al. (2012)
	AABB	2 RILs	293	–	20	2840.8	9.70	Gautami et al. (2012a)
	AABB	2 RILs	324	–	21	1352.1	4.17	Qin et al. (2012)
	AABB	10 RILs and 1 BC	897	–	20	3863.6	4.31	Gautami et al. (2012b)
	AA, BB & AABB	3 RILs	3693	–	20	2651	0.72	Shirasawa et al. (2013)

(Halward et al. 1993; Milla 2003; Moretzsohn et al. 2005; Nagy et al. 2012). These maps had 10–12 linkage groups (LGs) with map distance ranging from 1063 to 1231 cM, and 3.88 to 10.47 cM inter-marker distance. Subsequently, by using BC_1F_1 population for the diploid (AA genome), a genetic map was developed with 206 RFLP and RAPD marker loci spanning 800 cM distance and 3.88 cM inter-marker distance (Garcia et al. 2005). Later, two more genetic maps were constructed using the F_5 generation with the AFLP, RFLP, SSR, SCAR, SNP and SSCP markers and achieved 544–705.10 cM map distance and 1.24 to 1.84 cM inter-marker distance (Shirasawa et al. 2013; Bertioli et al. 2014). These genetic maps played an important role in several genetic studies including development of peanut A-genome assembly.

For the diploid (BB genome), initially F_2 population was used to construct SSR-based genetic map with 10–16 LGs which covered the map distance ranging from 1278.6 to 1294 cM and inter-marker distances 2.84 to 8.68 cM, respectively (Moretzsohn et al. 2009; Guo et al. 2012). Later in F_6 generation map was constructed with the 461 SSR and transposon markers and inter-marker distance was reduced up to 0.68 cM (Shirasawa et al. 2013).

7.4.2 Tetraploid Genetic Maps

Most of the linkage map construction work was done for the tetraploid genome by considering various mapping populations such as F_2, BC_1F_1 and recombinant inbred lines (RILs). In the F_2 population, several marker systems were deployed to construct maps viz. AFLP, SRAP, SSR, DArT, DArTseq and transposon, and achieved map distance up to 3526 cM with the inter-marker distance of 1.7–11.6 cM (Herselman et al. 2004; Wang et al. 2012, 2013; Shirasawa et al. 2012b; Chen et al. 2016b, Vishwakarma et al. 2016). Using BC_1F_1 population, two genetic maps were constructed with 298 RFLP and 370 SSR marker loci spread over 1844 and 2210 cM, respectively (Burow et al. 2001; Foncéka et al. 2009). Considering the importance of immortal population for high-resolution mapping, several maps were prepared in the RIL populations. With the SSR, CAPS, SNP and transposon markers, 29 to 1685 loci were mapped on 8–26 LGs, map density and inter-marker distance of these map were 402–2208 cM and 0.8–18.5 cM, respectively (Jiang et al. 2007; Hong et al. 2008, 2009; Varshney et al. 2009b; Peng et al., 2010; Ravi et al. 2011; Sarvamangala et al. 2011; Hong et al. 2010; Khedikar et al. 2010; Sujay et al. 2012; Gautami et al. 2012b; Qin et al. 2012; Mondal et al. 2012; Zhou et al. 2014; Zhao et al. 2016). Recently, using NGS-based ddRADseq technique, Zhou et al. (2014) provided a well-saturated map for the tetraploid peanut and mapped 1685 marker loci, including 1621 SNPs and 64 SSR markers spanning a distance of 1447 cM with the average distance of 0.9 cM. Use of DArT and DArTseq based genotyping resulted in development of three genetic maps using F_2 populations with 854 loci (ICGV 07368 × ICGV 06420; Shasidhar et al. 2017), 1152 loci (ICGV 00350 × ICGV 97045; Vishwakarma et al. 2016) and 1435 loci (ICGV 06420 × SunOleic 95R; Shasidhar et al. 2017).

The first SSR-based genetic map was developed using a RIL population (TAG 24 × ICGV 86031) with 135 loci covering 1270.5 cM map distance (Varshney et al. 2009b). This genetic map was then further saturated to 191 SSR mapped loci covering 1785.4 cM genome distance (Ravi et al. 2011). Subsequently, other two SSR-based genetic maps were prepared with 56 (462.24 cM; TAG 24 × GPBD 4; Khedikar et al. 2010), and 45 marker loci (657.9 cM; TG 26 × GPBD 4; Sarvamangala et al. 2011). Later, these maps were saturated to 188 (1922.4 cM) and 181 (1963 cM) marker loci, respectively (Sujay et al. 2012). Two more RIL populations derived from the cross ICGS 44 × ICGS 76 and ICGS 76 × CSMG 84–1 were used for genetic map construction with 82 (831.4 cM) and 119 (2208.2 cM) marker loci, respectively (Gautami et al. 2012a). In addition to individual genetic maps, different maps were combined to construct integrated or consensus genetic maps. Genetic mapping information from two RIL mapping populations (TAG 24 × GPBD 4 and TG 26 × GPBD 4) segregating for foliar disease resistance

were used for constructing the first consensus map with 225 SSR loci covering total map distance of 1152.9 cM (Sujay et al. 2012). The second consensus map was developed using three RIL populations (TAG 24 × ICGV 86031, ICGS 44 × ICGS 76 and ICGS 76 × CSMG 84-1) segregating for drought tolerance related traits, and mapped 293 marker loci LGs (2840.8 cM) (Gautami et al. 2012a). In an international effort, reference consensus genetic map was prepared by using 10 RILs and one backcross (BC) populations with 897 marker loci on 20 LGs spanning a map distance of 3863.6 cM with an average map density of 4.4 cM (Gautami et al. 2012b). This consensus map was further improved by adding five more populations and achieved a dense consensus genetic map with 3693 marker loci covering 2651 cM distance (Shirasawa et al. 2013).

7.5 Trait Mapping Through Linkage Mapping

Initial trait mapping work started with the RAPD markers to identifying linked markers with the root-knot nematode resistance (Garcia et al. 1996; Burow et al. 1996). The RAPD markers were not preferred due to several technical problems. Nevertheless, few of these were later converted to sequence characterized amplified region (SCAR) markers for deploying in genetic and breeding studies (Chu et al. 2007). Other studies included AFLP, SSR and SNP markers for establishing an association with resistance to groundnut rosette disease and *Sclerotinia* blight, and oil quality traits namely oleic acid and linoleic acid (Herselman et al. 2004; Chenault and Maas 2006 and Lopez et al. 2000). More efforts were initiated to dissect important agronomic traits with latest NGS-based technology like GBS and array-based genotyping in peanut. Nonetheless, to achieve the sustainable yield of crop breeding for several biotic and abiotic stresses is mandatory. Handful genomics tools and techniques provided breeders a new way to dissect useful QTLs/genes leading to their deployment in breeding (Janila et al. 2016b; Pandey et al. 2016a). In total, 46 major QTLs were identified for several biotic stresses with the phenotypic variation explained (PVE) 10.05–82.96%, 59 for the abiotic stresses and their related traits with the PVE range of 10.0–22.24%, 50 major QTLs for agronomic and yield component traits with the PVE range of 10.1–33.36% and 50 major QTLs for other morphological traits with 10.0–28.2% PVE and 60 major QTLs for seed and oil quality traits with the PVE range of 10.2–45.63% (Table 7.2).

In case of peanut, several biotic stresses affect yield and quality adversely including rust, ELS and LLS, nematode, GRD, TSWV, bacterial wilt, *Sclerotinia minor*, *Aspergillus* and aflatoxin contamination. For rust resistance, 18 major QTLs were reported with 10.68–82.96% PVE (Khedikar et al. 2010; Sujay et al. 2012; Mondal et al. 2014a; Leal-Bertioli et al. 2015). Similarly, 15 major QTLs for LLS resistance with the PVE range of 10.27–67.98%, 4 major QTLs for GRV resistance with the PVE range of 10.05–76.1%, 5 major QTLs for TSWV with the PVE range of 10.64–35.8%, 2 major QTLs for bacterial wilt resistance with the PVE range of 12–22% and 13 major QTLs for nematode resistance with the PVE range of 11.9–22.1% (Herselman et al. 2004; Liang et al. 2009; Sujay et al. 2012; Qin et al. 2012; Wang et al. 2013; Burow et al. 2014; Zhao et al. 2016; Leal-Bertioli et al. 2016). For aflatoxin contamination (AC), so far only three major QTLs were identified with 10.5–22.7% PVE (Liang et al. 2009). In addition to this, Mondal et al. (2014b) identified QTLs for the Bruchid resistance component traits.

Among the abiotic stresses, heat and terminal drought are the two major stress factors causing severe yield loss and quality deterioration of the produce in peanut in addition to other factors such as sodic and acidic nature of soil, micronutrients deficiency (Zinc, Iron) and aluminium toxicity (Janila and Nigam 2013). In peanut, ICRISAT with the research partners has done pioneer work to identify linked markers for drought tolerance related traits. In this context, major QTLs were identified successfully for transpiration (5 QTLs), transpiration efficiency (4 QTLs), carbon discrimination ratio (1 QTL), specific leaf area (6 QTLs), leaf area (1 QTL), SPAD chlorophyll

Table 7.2 List of major QTLs identified for important traits in peanut

S. N.	Traits studied	Major QTLs identified	Phenotypic variation explained (%)	References
Biotic stress resistance				
1	Late leaf spot (LLS)	20	10.3–68.0	Sujay et al. (2012), Wang et al. (2013), Pandey et al. (2016b, 2017b)
2	Leaf rust	18	10.7–83.0	Khedikar et al. (2010), Sujay et al. (2012), Mondal et al. (2014a), Leal-Bertioli et al. (2015), Pandey et al. (2016b)
3	Resistance to *Aspergillus flavus* invasion	3	10.5–22.7	Liang et al. (2009)
4	Aphid vector of groundnut rosette disease	4	10.1–76.1	Herselman et al. (2004)
5	Resistance to tomato spotted wilt virus (TSWV)	6	10.6–35.8	Qin et al. (2012), Wang et al. (2013), Pandey et al. (2017b)
6	Root-knot nematode	13	11.9–22.1	Burow et al. (2014), Leal-Bertioli et al. (2016)
7	Bacterial wilt (BW)	4	12.0–22.0	Peng et al. (2010), Zhao et al. (2016)
8	Bruchid resistance component traits	10		Mondal et al. (2014b)
Abiotic stress tolerance				
9	Transpiration (T)	5	10.3–18.2	Varshney et al. (2009b), Ravi et al. (2011), Gautami et al. (2012a)
10	Transpiration efficiency (TE)	4	12.3	Ravi et al. (2011), Gautami et al. (2012a)
11	Specific leaf area (SLA)	9	11.0–20.3	Varshney et al. (2009b), Ravi et al. (2011)
12	Leaf area (LA)	1	11.5	Ravi et al. (2011)
13	SPAD chlorophyll metre reading (SCMR)	17	10.6–31.2	Varshney et al. (2009b), Ravi et al. (2011)
14	Biomass	3	15.6–20.3	Ravi et al. (2011)
15	Canopy conductance (ISC)	3	11.9–22.2	Ravi et al. (2011)
16	Total dry matter (TDM)	1	22.4	Gautami et al. (2012a)
17	Harvest index	1	18.1	Fonceka et al. (2012)
18	Hundred pod weight	2	15.0–17.0	Fonceka et al. (2012)
19	Hundred seed weight	2	12.4–14.9	Fonceka et al. (2012)
20	Haulm weight	2	13.5–17.5	Fonceka et al. (2012)
21	Pod number	2	9.6–12.6	Fonceka et al. (2012)
22	Total biomass	2	11.0–16.6	Fonceka et al. (2012)

(continued)

Table 7.2 (continued)

S. N.	Traits studied	Major QTLs identified	Phenotypic variation explained (%)	References
23	Stress tolerance indices (STI)—Hundred pod weight	2	13.9–16.8	Fonceka et al. (2012)
24	STI—Hundred seed weight	2	15.5–16.2	Fonceka et al. (2012)
25	STI—Haulm weight	2	16.4–17.1	Fonceka et al. (2012)
26	STI—Pod number	2	10.4–19.4	Fonceka et al. (2012)
27	STI—Pod weight	1	12.3	Fonceka et al. (2012)
28	STI—Seed number	6	11.0–26.0	Fonceka et al. (2012)
29	STI—Seed weight	5	11.5–15.2	Fonceka et al. (2012)
30	STI—Total biomass	2	10.8–20.1	Fonceka et al. (2012)
Agronomic and yield component traits				
31	Shoot dry weight (ShDW)	2	14.4–22.1	Gautami et al. (2012a)
32	Haulm weight	2	10.4–36.1	Ravi et al. 2011, Fonceka et al. (2012)
33	Harvest index	2	11.0–40.1	Gautami et al. (2012a), Fonceka et al. (2012)
34	Pod mass/plant	3	13.1–18.3	Liang et al. (2009)
35	Mature pods/plant	2	11.9–12.3	Liang et al. (2009)
36	Pod number	1	14.2	Fonceka et al. (2012)
37	Number of branches	3	10.2–17.3	Liang et al. (2009)
38	Number of fruit branches	1	17.5	Liang et al. (2009)
39	Height of main axis	3	10.3–12.8	Liang et al. (2009)
40	Stem diameter	2	10.4–24.1	Liang et al. (2009)
41	Leaf length, width and length/width ratio	7	12.4–18.9	Liang et al. (2009)
42	Length of main stem	2	15.7–19.2	Shirasawa et al. (2012b)
43	Length of the longest branch	2	14.2–21.1	Shirasawa et al. (2012b)
44	Number of branches	1	15.6	Shirasawa et al. (2012b)
45	Weight of plant	1	11.8	Shirasawa et al. (2012b)
46	Weight of mature pod per a plant	1	28.1	Shirasawa et al. (2012b)
47	Weight of seeds	1	19.1	Shirasawa et al. (2012b)
48	Yield parameters	5	10.1–17.7	Selvaraj et al. (2009)
49	Hundred pod weight	2	15.1–20.6	Fonceka et al. (2012)
50	Hundred seed weight	2	15.7–16.3	Fonceka et al. (2012)
51	Pod weight	1	11.7	Fonceka et al. (2012)
52	Shell weight	1	12.6	Fonceka et al. (2012)

(continued)

Table 7.2 (continued)

S. N.	Traits studied	Major QTLs identified	Phenotypic variation explained (%)	References
53	Seed number	1	14.5	Fonceka et al., 2012
54	Seed weight	1	11.0	Fonceka et al. (2012)
55	Total biomass	1	13.2	Fonceka et al. (2012)
Other morphological traits				
56	Flowering date	1	19.5	Shirasawa et al. (2012a, b)
57	Angle of branch	2	11.9–23.2	Shirasawa et al. (2012a, b)
58	Constriction of pod	7	10.0–23.9	Shirasawa et al. (2012a, b), Fonceka et al. (2012)
59	Pod beak	5	11.6–17.4	Fonceka et al. (2012)
60	Pod length	5	20.5–28.2	Shirasawa et al. (2012b), Chen et al. (2016b)
61	Thickness of pod	1	21.7	Shirasawa et al. (2012a, b)
62	Pod width	8	15.2–25.5	Shirasawa et al. (2012a, b), Fonceka et al. (2012), Chen et al. (2016b)
63	Seed length	8	11.2–20.8	Fonceka et al. (2012), Chen et al. (2016b)
64	Seed width	4	14.2–23.7	Fonceka et al. (2012), Chen et al. (2016b)
65	Growth habit	5	13.9–17.3	Fonceka et al. (2012)
66	Main stem height	4	10.0–26.7	Fonceka et al. (2012)
Seed and oil quality				
67	Oil content	6	10.2–14.2	Selvaraj et al. (2009), Sarvamangala et al. (2011), Pandey et al. (2014c)
68	Protein content	3	10.2–13.4	Liang et al. (2009), Sarvamangala et al. (2011)
69	Carbon discrimination ratio	1	12.2	Ravi et al. (2011)
70	Oleic acid	9	10.7–38.4	Pandey et al. (2014c)
71	Linoleic acid	8	12.0–39.5	Pandey et al. (2014c)
72	Oleic/linoleic (O/L) acid ratio	3	10.8–45.6	Pandey et al. (2014c)
73	Palmitic acid	6	10.6–37.4	Wang et al. (2014)
74	Stearic acid	6	17.8–40.57	Wang et al. (2014)
75	Arachidic acid	4	28.3–36.9	Wang et al. (2014)
76	Gadoleic acid	9	11.2–26.1	Wang et al. (2014)
77	Behenic acid	2	12.4–13.6	Wang et al. (2014)
78	Lignoceric acid	3	10.0–12.6	Wang et al. (2014)
79	Total phenolics	1	12.5	Mondal et al. (2015)
80	Total flavonoids	5	25.0–67.0	Mondal et al. (2015)
81	DPPH radical scavenging	4	11.5–33.0	Mondal et al. (2015)

metre reading (SCMR) (12 QTLs), biomass (3 QTLs), shoot dry weight (2 QTLs), haulm weight (1 QTL), harvest index (1 QTL), canopy conductance (3 QTLs) and total dry matter (1 QTL) (Varshney et al. 2009b; Ravi et al. 2011; Gautami et al. 2012a). The another study while dissecting drought tolerance traits Fonceka et al. (2012) identified two major QTLs each for 100 pod weight, 100 seed weight, haulm weight, pod number, total biomass, STI-100 pod weight, STI-100 seed weight, STI-haulm weight, STI-pod number, STI-total biomass, one major QTL each for STI-pod weight, STI-seed number and STI-seed weight. Although, above-mentioned studies provided preliminary idea on the probable genomic regions showing association with drought tolerance related traits, but still no linked marker has been validated so far which can be deployed in breeding. Nevertheless, lots of genetic populations have been developed while conducting above studies. Generation of multi-location and multi-replicated phenotyping and high throughput genotyping data on these genetic populations will facilitate high-resolution trait mapping and identification of linked markers for drought tolerance related traits.

7.6 Oil and Nutritional Quality

In most populated countries like China and India, peanut is mainly crushed for oil extraction while it mainly serve as table purpose and preferred for low oil content in other countries like United States of America. For oil content, 6 major QTLs were identified with the PVE ranging from 10.2 to 14.18% (Selvaraj et al. 2009; Sarvamangala et al. 2011; Pandey et al. 2014b). For protein content, to date only two workers namely Liang et al. (2009) and Sarvamangala et al. (2011) separately reported three major QTLs with PVE ranging from 10.7% to 13.4%.

Peanut oil contains unsaturated fatty acids (UFA), i.e. oleic and linoleic acid and saturated fatty acids (SFA), i.e. palmitic acid, stearic acid, arachidic acid, behenic acid, lignoceric acid and gadoleic acid. The UFAs were further categorized into monounsaturated fatty acid (MUFA) such as oleic acid (C18:1), and polyunsaturated fatty acid (PUFA), i.e. linoleic acid (C18:2). Oleic acid is known to diminish the risk of cardiovascular disease (CVD) by decreasing the levels of serum low-density lipoproteins (LDL) cholesterol and preserving the levels of high-density lipoproteins (HDL). The ratio of UFAs in peanut oil comprises 80% while remaining 20% accounted by SFAs, apart from this UFA is also very high in peanut oil in comparison to the butter, coconut oil and palm oil that bestow peanut oil as a healthier food as a consuming oil (Johnson et al. 2009). With the addition of a double bond to C18:1 (oleic acid), oleic acid converts into linoleic acid, and this reaction is catalyzed by the fatty acid desaturase (FAD) enzyme (Ray et al. 1993). Genetics studies revealed that the high oleic acid is controlled by two homozygous recessive mutant alleles of FAD2A and FAD2B genes. The first study of reporting QTLs for oleic acid, linoleic acid and oleic/linoleic (O/L) ratio other than FAD2A and FAD2B reported 20 major QTLs with the PVE range 10.71–45.63 (Pandey et al. 2014b). Another study reported 30 major QTLs for saturated fatty acids (Wang et al. 2015). In addition to this, ten major QTLs were reported with the PVE% range 12.5, 25–67 and 11.5–33 for the total phenolics, total flavonoids and DPPH radical scavenging, respectively by the Mondal et al. (2015). The linked markers are available for use in breeding to improve the fatty acid profiles in peanut.

7.7 Agronomic and Morphological Traits

Enhancing the pod yield has been the main goal since the first day of breeding and will remain the main goal even in future seeing the unprecedented population growth globally. In this context, efforts were made to identify the QTLs associated with yield and yield component traits. So far, a total 50

QTLs were reported for yield and yield component traits with PVE range 10.1–40.1% (Selvaraj et al. 2009; Liang et al. 2009; Fonceka et al. 2012; Shirasawa et al. 2012b). Major QTLs for flowering date, angle of branch, pod characteristics such as constriction, beak, length, thickness and width, seed width and length, pod width and length, growth habit and main stem height were identified in three separate studies (Fonceka et al. 2012; Shirasawa et al. 2012b; Chen et al. 2016b). In addition to bi-parental populations, genetic populations involving multiple parents (such as MAGIC and NAM) have been developed by ICRISAT for conducting high-resolution mapping using multi-location and multi-replicated phenotyping and high throughput genotyping data on these genetic populations.

7.8 Trait Mapping Through Linkage Disequilibrium/Association Mapping

Bi-parental populations have limitations for being able to provide allelic variation for few traits and enable to dissect a small fraction of the probable alleles through linkage mapping. Furthermore, genetic resolution of QTL mapping often remains limited in a range of 10–30 cM due to confined number of meiotic events that are captured in a bi-parental mapping population (Zhu et al. 2008). Globally, availability of large number of peanut germplasm provided opportunity to think out of box and utilize this germplasm in trait mapping using association mapping approach. To exploit these available set of germplasm, association mapping approach for trait mapping is very promising (Gupta et al. 2014). Association mapping can be categorized in two categories candidate gene-based association (CGAS) and genome-wide association mapping (GWAS) (Zhu et al. 2008).

Phenotyping for quality traits and genotyping of the US 'Mini Core Collection' with 81 SSR markers identified two functional SNP markers for two fatty acid desaturase (*FAD2* for oleic acid, linoleic acid and oleic-to-linoleic ratio (Wang et al. 2011). Subsequently in another study, marker-trait associations (MTAs) with low phenotypic variation (1.05–4.81% PVE) for 15 agronomic traits were identified in in Chinese 'Mini-Core Collection' (Jiang et al. 2014). Recently, at ICRISAT in order to identify MTAs, phenotyping and genotyping data (4597 polymorphic DArT loci and 154 SSR loci) generated on 'Reference Set' developed by ICRISAT comprising of 300 accessions were used for association analysis and identified 524 highly significant markers with 5.8–90.1% PVE for 36 traits (Pandey et al. 2014b). Recently, 'Reference Set' with 300 accessions, US 'Mini Core Collection' containing 112 accessions, and Chinese 'Mini Core Collection' with 298 accessions, an endeavour has been initiated at International level to generate high throughput genotyping data in addition to precise phenotyping to conduct high-resolution association analysis for identifying linked markers and accessions with superior alleles for use in the breeding programmes.

7.9 Advanced Backcross (AB) QTL Mapping

Wild species of peanut is a reservoir of useful genes and alleles for biotic and abiotic stresses (Upadhyaya et al. 2012). These genes were untouched and unexploited throughout the course of evolution and domestication. Despite owns boastful wild germplasm, just few (1.1%) were used to develop advanced breeding lines (Sharma et al. 2013). Recently, through remarkable attempts, some elite cultivars with multiple disease resistance were released from ICRISAT and USA (Sharma et al. 2013; Burow et al. 2013). Similar to other polyploid species, continuous domestication of cultivated peanut narrowed the genetic diversity which impose a genetic bottleneck. Since direct introgression of the useful genes from the wild species to cultivated gene pool is very difficult, the synthetic amphidiploid were used as bridge species to defeat the reproductive barriers between the wild diploids and the cultivated species. At ICRISAT, 17 new synthetic amphiploids and autotetraploids populations were developed to broadening genetic

base (Mallikarjuna et al. 2012; Shilpa et al. 2013). These new synthetics were also reported as resistance to late leaf spot and peanut bud necrosis disease.

To save the time and enhance the accuracy during identification and introgression of useful alleles from wild to cultivated genotypes, molecular markers proved as a very handful tool. In this context, strategy like advanced-backcross QTL (AB-QTL) has been suggested by the Tanksley et al. (1996). In this strategy, identification of linked markers as well as introgression of trait of interest can be done simultaneously. In this direction, 110 QTLs were identified for several traits including physiological, agronomic, morphological traits and resistance to the root-knot nematode (Fonceka et al. 2012; Burow et al. 2014). Further, at ICRISAT, two AB-QTL populations were developed and phenotyped for several disease resistance traits. The genotyping data (DArT markers) and phenotyping data were analysed leading to identification of QTLs for rust and LLS resistance (Varshney et al. 2013). Two of the above-mentioned synthetics namely ISATGR 278-18 (*A. duranesis* × *A. batizocoi*) and ISATGR 5B (*A. magna* × A. *batizocoi*) were used to introgress foliar disease resistance in five elite Indian peanut varieties namely ICGV 91114, ICGS 76, ICGV 91278, JL 24, and DH 86 using backcross breeding approach (Kumari et al. 2014). In addition to disease resistance, these lines have also shown range of variation for other important morphological and agronomic traits.

7.10 Next-Generation Genetic Populations for High-Resolution Trait Mapping

With the advancements in the NGS technology which can generate huge genomic sequence data in very short time. Several analysis softwares/tools have become available in public domain for analysing large data sets. These developments have allowed us to develop even more complex and larger genetic populations to perform high-resolution trait mapping. Some of these important populations include MAGIC, NAM and recombinant inbred advanced intercross line (RIAIL) populations (Morrell et al. 2012). It is important to note that MAGIC population facilitate increased recombination events by making multiple crosses using multiple parents to create highly diverse genetic population for conducting high-resolution genetic mapping (Cavanagh et al. 2008). Analysis of such population has remained challenge due to presence of multiple alleles coming from different founder parental genotypes in the population. Nevertheless, a whole-genome average interval mapping (WGAIM) approach has been developed recently for conducting QTL analysis (Verbyla et al. 2014). This approach is very promising for conducting high-resolution trait mapping for several traits simultaneously. Recently, at ICRISAT two MAGIC populations for (aflatoxin resistance and drought tolerance) and two MAGIC population for agronomic traits have been developed. NAM is another promising approach to dissect the genetic basis of complex traits by capturing genetic diversity of selected diverse parents (founders). Most importantly, the NAM population has higher power QTL detection as compared with bi-parental mapping populations (Yu et al. 2008; McMullen et al. 2009). In peanut, two NAM populations, i.e. one each for Spanish (ICGV 91114 and 22 testers) and Virginia type (ICGS 76 and 21 testers) were developed (Varshney 2016; Pandey et al. 2016a). The development of MAGIC and NAM populations have given birth to a new method of trait mapping called joint linkage-association mapping (JLAM). These populations can be used to conduct linkage as well as association mapping and are very useful for conducting high-resolution mapping (Gupta et al. 2014).

7.11 Emerging NGS-Based Trait Mapping Strategies

It has been discovered that a high level of resolution can be achieved with the help of high-density genotyping by using NGS methods

(Huang et al. 2009) and mapping by sequencing (Schneeberger and Weigel 2011). Recently, few trait mapping approaches demonstrated speedy detection of genomic regions and candidate genes controlling the targeted traits such as MutMap, QTL-seq and BSR-seq.

Abe et al. (2012) identified successfully causal mutations for pale green leaves and semi-dwarfism in rice using MutMap approach. In this approach, whole-genome re-sequencing (WGRS) is performed for the pooled DNA samples from a F_2 segregating progeny of a cross between a mutant and its wild type (WT). The concept of SNP identification is based on the SNP index and the sequence data of bulked DNA is aligned with the reference sequence. The SNPs with sequence reads containing only of the mutant sequences (SNP index = 1) are considered to be linked to the causal SNP for the mutant phenotype. The MutMap is theoretically similar to some of other related methods such as SHOREmap (Schneeberger et al. 2009) and next-generation mapping (Austin et al. 2011). The same group has updated MutMap to MutMap plus where the same concept of identification of causative SNP for the mutant phenotype can be achieved without crossing the mutant with WT line. Therefore, the DNA of M_3 progenies with extreme phenotypes are bulked to get the SNP index (Fekih et al. 2013). To overcome the difficulty of mutations in the missing genomic regions from the reference (gap) genome when the reference genome is aligned to the re-sequenced lines, Takagi et al. (2013a) has proposed MutMap-Gap strategy where MutMap is used to identify the causal SNP followed by de novo assembly, alignment, and identification of the causal mutation within the genome gaps. In peanut, this strategy can be implemented too for the identification of agronomic traits of interest.

In the QTL-seq technique, MutMap strategy was conceptually integrated to the normal F_2 and RIL population (Takagi et al. 2013b). The principle involves a combination of bulked segregant analysis and whole genome re-sequencing for rapid identification of agronomically important QTLs. After alignment of the sequence with reference sequence, SNP index is derived to narrow down to the causal SNP (Takagi et al. 2013b). This strategy has been used to identify an early flowering QTL in cucumber (Lu et al. 2014). In peanut, by using QTL-seq approach three for rust resistance and one for LLS resistance allele-specific diagnostic markers were identified (Pandey et al. 2016b). These results prove the usefulness of QTL-seq approach for the rapid and precise and identification of candidate genomic regions and development of diagnostic markers for breeding applications.

BSR-sequencing is one of the potential methods where the candidate genes/markers associated with the trait can be identified at the expression level. DNA-based BSA requires access to quantitative genetic markers that are polymorphic in the mapping population. The modification over BSA method, BSR-Seq makes use of RNA-Seq reads to efficiently map genes even in populations for which no polymorphic markers have been previously identified (Liu et al. 2012). In this approach, it is possible to conduct de novo SNP discovery and quantitatively genotype the bulks from extreme phenotype or mutants using appropriate computational tools. This is relatively new technique that is less explored in plants and there are no published reports yet in peanut. As this approach uses the expression data into consideration, there is advantage of identifying probable candidate genes to dissect important traits.

7.12 Molecular Breeding for the Disease Resistance and Oil Content and Quality Traits

The identification of molecular markers linked to desirable traits in peanut has provided the pace to the peanut improvement programs using molecular breeding. Two molecular breeding approaches namely marker-assisted backcrossing (MABC) and marker-assisted selection (MAS) facilitate transfer QTLs/gene from source genotype to elite recipient cultivars. The MABC

and MAS approaches are very precise in selection at very initial stage of the plant through the trait linked markers. Additionally, MABC approach shortens the generation to achieve higher recurrent parent genome recovery as compared to conventional breeding methods. Some of the successful examples of MABC/MAS application and their output have been discussed below.

At the earliest through MABC approach, Simpson et al. (2003) developed Nematode resistance lines and registered as 'NemaTAM' variety. Chu et al. (2011) pyramided high oleic acid and nematode resistance in cultivated peanut and also developed the CAPS markers for *ahFAD2A* and *ahFAD2B* mutant alleles responsible for oil quality traits. The South African peanut cultivars were improved for the high oleic acid trait through MAS (Mienie and Pretorius, 2013). In Indian continent, rust and LLS are the major foliar fungal diseases of peanut causing 40–70% losses in pod yield. Most of the popular cultivars in major growing state viz. Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh have been reported susceptible to rust and LLS. With an objective to breed resistant varieties for foliar disease resistance, Varshney et al. (2014) introgressed one major QTL each for rust resistance and LLS resistance conferring >80% and 67.98% PVE, respectively, in the popular varieties namely ICGV 91114, JL 24 and TAG 24. Furthermore recently, Janila et al. (2016c) evaluated these selected introgression lines at three locations including disease hot spots regions of India. The reason was to assure the expression of resistance governed by the QTL region, as different factors viz. genotype background, environment and genotype × environment interactions work behind this. Resultantly, six best ILs 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. Pod yield increase was contributed by increase in seed mass and number of pods per plant. The most interesting result was combining short maturity duration together with foliar disease resistance through MABC approach which was not earlier achieved through conventional breeding approaches. Similarly, for improving quality traits, MABC/MAS approaches were used to improve three major fatty acids namely oleic, linoleic and palmitic acids by transferring two mutant alleles from donor 'SunOleic 95R' in three Indian elite varieties namely ICGV 06110, ICGV 06142 and ICGV 06420 (Janila et al. 2016a). Now the efforts are underway to combine foliar disease resistance and oil quality through marker-assisted gene pyramiding approach.

7.13 Conclusion

Peanut is a crop of global importance and is an essential component of human food basket. This crop has been lacking optimal genomic resources to improve the breeding efficiency for achieving higher genetic gain in less time. The year '2016' has been very good as genome assemblies for both the diploid ancestors of cultivated peanut were made available. Last couple of years were also good in the context of developing several genetic populations and preliminary genetic mapping and trait mapping. It is equally important that now peanut is also witnessing development of high throughput genotyping platforms and high-resolution multi-parent mapping populations. The availability of such resources will further accelerate development and deployment of genomic resources targeting peanut genetic improvement.

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