Chapter 12
Groundnut

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Abstract Groundnut or peanut is an important legume nut known for its multifarious uses including oil production, direct human consumption as food and also animal consumption in the form of hay, silage and cake. Being a grain legume, peanut has an important nutritional value for human beings, and its nutritional value has been exploited for combating malnutrition in children. The breeding objectives in groundnut focus on increasing yield, incorporating resistance/tolerance to biotic and abiotic stresses and improving oil and nutritional quality including safety of its consumption by humans and animals. However, limited genetic variability in the cultivated germplasm and difficulties in hybridisation have slowed down the progress in groundnut breeding. The wild relatives are considered as sources of several agriculturally important traits including resistance to pests and pathogens, tolerance to abiotic stresses and variable nutritional value. These resources have been used in groundnut breeding programmes for improving the above traits, simultaneously addressing the constraint of reproductive barrier in successful hybridisation arising due to different ploidy levels of *A. hypogaea* and its wild relatives. This has been achieved through different routes: the hexaploid pathway, two different diploid/tetraploid pathways and genetic engineering-based methods. Nonetheless, the use of wild introgressions in groundnut improvement programmes has not been up to the desired extent, and therefore concerted efforts for a large-scale generalised introgression programme are required. This chapter discusses the evaluation and utilisation of alien introgressions in groundnut improvement, the achievements made hitherto and the future strategies for initiating a large-scale introgression programme.

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12.1 Introduction

Groundnut, also commonly known as peanut (*Arachis hypogaea*), is a tropical legume mainly grown to produce oil and for human and animal consumption. Peanut is grown in about 120 countries in the world in a total area of 24.6 million ha, with a world production of 38.2 million tonnes (Mt). Asia is the major peanut-producing region in the world. In this region, China and India are the major contributors with 15.7 and 5.6 Mt in 2010, respectively (FAOSTAT 2010). Africa ranks second in the world peanut production. In this region, Nigeria (2.6 Mt), Senegal (1.2 Mt) and Sudan (0.7 Mt) are the major producing countries (FAOSTAT 2010). In Africa and Asia peanut is mainly grown by resource-poor farmers. In the Americas, the USA and Argentina are the major producing countries with 1.8 and 0.6 Mt in 2010, respectively.

Peanut yields vary drastically between regions and between countries within a region. Although Africa is the second region in terms of production, it has the lowest yield (1 t/ha on average) as compared to Asia (1.8 t/ha) and to the Americas (3 t/ha). In West Africa, peanut yields vary from 0.5 t/ha in Niger to 1 t/ha in Senegal and can reach up to 1.5 t/ha in Nigeria. In Asia yields vary from 1.5 t/ha in India to 3 t/ha in China. The low peanut yields observed in many countries in Africa and Asia are related to rainfed and low-input growing conditions. In these countries where the rainfall pattern is irregular, peanut is often subjected to drought.

Worldwide peanut production is principally dedicated to oil and food products. Between 1996 and 2000, 49 % of world production has been used for oil and 41 % as food product components (Revoredo and Fletcher 2002). Peanut is also used for feed through the valorisation of oil cakes that represent an interesting source of proteins for livestock. In most Sahelian countries, groundnut straw is also used as dried hay and represents a major source for cattle feed during the dry season. As is the case with most of the grain legumes, peanut has an important nutritional value for human consumption. Several studies have reported a positive impact of peanut on human health, and its nutritional value has been exploited for the elaboration of highly nutritious food products used in the treatment of severe child malnutrition (Briend 2001).

Peanut breeding objectives are mainly focused at increasing yield and improving resistance to foliar diseases and nematodes, tolerance to drought, quality of oil and food and safety (resistance to aflatoxin contamination and reduced allergenicity). Significant progress has been achieved in developing elite cultivars using sources of adaptive traits and disease resistance that exists in cultivated germplasm collections. This was particularly the case for drought tolerance-related traits, oil quality and resistance to rosette disease. However, for some other traits such as resistance to early and late leaf spot, rust and nematode, only moderate levels of resistance are observed in the cultivated germplasm (Holbrook and Stalker 2003).

Peanut breeding has also been slowed down by the difficulties in making large numbers of crosses and by the low number of progenies produced per cross. This has limited the exploration and the utilisation of cultivated genetic resources. In addition to these practical constraints, and in spite of the morphological variability that is observed in the cultivated gene pool, there are limitations to genetic improvement that can be achieved using only cultivated germplasm. A clear example of this is disease resistance: wild species display much stronger disease resistances than are found in cultivated peanut. There are also good theoretical reasons to believe that genetic limits for more complex traits like yield and drought tolerance can be overcome by using wild relatives of the crop as this has been the case in other crops (Gur and Zamir 2004). For these reasons, peanut breeders have for many years been interested in the introduction of new alleles from wild species.

12.2 Peanut Gene Pools and Genetic Resources

The genus *Arachis* consists of 80 described species (Krapovickas and Gregory 1994, 2007; Valls and Simpson 2005) and is divided into nine taxonomic sections: *Trierectoides*, *Erectoides*, *Procumbentes*, *Rhizomatosae*, *Heteranthae*, *Caulorrhizae*, *Extranervosae*, *Triseminatae* and *Arachis* (Fig. 12.1). These divisions were made

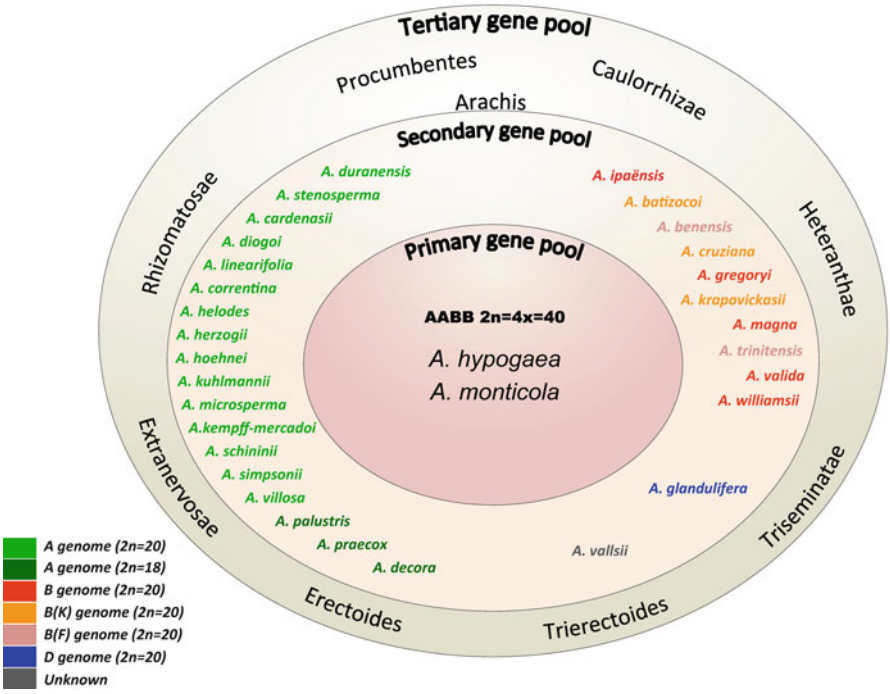

Fig. 12.1 Primary, secondary and tertiary gene pools of the genus *Arachis*

based on sexual compatibilities, morphological and cytogenetic features and geographic distributions (Krapovickas and Gregory 1994). The sexual compatibility data available from a large number of crossing experiments is very informative as to the barriers between gene pools in the genus (Krapovickas and Gregory 1994). The section *Arachis* contains the primary gene pool of cultivated peanut with two tetraploids, *A. hypogaea* and *A. monticola*. ($2n=4x=40$; genome AB) and the secondary gene pool with the most closely related wild species.

Arachis hypogaea presents considerable morphological variation, and two subspecies, *hypogaea* and *fastigiata*, have been described (Krapovickas and Gregory 1994). Subspecies *hypogaea* has spreading growth habit with side branches procumbent to decumbent, a long growth cycle, no flowers on the central stem and regularly alternating vegetative and reproductive side stems. This subspecies is divided into two botanical varieties *hypogaea* and *hirsuta*, the latter being distinguished by more hirsute leaflets and even longer cycle. These varieties, respectively, exemplify "Runner" and "Peruvian Runner" agronomic classes. The subspecies *fastigiata* Waldron has a more erect growth habit with side branches erect to procumbent, a shorter cycle, flowers on the central stem and reproductive and vegetative stems distributed in a disorganised fashion. This subspecies is divided into four botanical varieties, *fastigiata*, *vulgaris*, *aequatoriana* and *peruviana*. The former two are by far the most economically important and exemplify the agronomic classes "Spanish" and "Valencia", respectively (Krapovickas and Gregory 1994).

Within the agronomic classes, modern cultivars are relatively uniform compared to landraces. Especially in South America, but also in Africa and Asia, landraces are spectacularly diverse. This diversity provides a source for constant study, such as the recent interesting description of 62 distinct landraces in Bolivia (Krapovickas et al. 2009). Also, new collections of landraces continue to be made. For instance, in the Xingu Indigenous Park in the Central-West of Brazil, the Kayabi people cultivate peanuts which are morphologically very diverse, displaying combinations of unusual characters which make them unique. Some types form very large plants and have a very long cycle; some have extremely large seeds. The different types also display diverse seed colours and patterns, purple, brown, red or white, variegated or uniform in colour (Freitas et al. 2007; Bertioli et al. 2011).

Morphological diversity is so high that a different origin for the two subspecies was proposed. This hypothesis was supported by the partial reproductive isolation of the two subspecies (Singh and Moss 1982; Lu and Pickersgill 1993). However, molecular data has firmly contradicted this hypothesis. Genetic variability observed among commercial cultivars and landraces of peanut is so low that it is generally accepted that peanut is an allotetraploid of recent and single origin (Halward et al. 1993; Kochert et al. 1996; Raina et al. 2001; Milla et al. 2005).

The secondary gene pool includes *A. hypogaea*'s most closely related wild species that can be used for peanut crop improvement. Most of these species are diploid ($2n=2x=20$) with metacentric chromosomes of similar size (genomes A, B, F and K); one species (*A. glandulifera*) is diploid with an asymmetric karyotype (genome D); three can be considered dysploid ($2n=2x=18$) (Krapovickas and Gregory

1994; Lavia 1998; Peñaloza and Valls 1997; Stalker 1991; Valls and Simpson 2005; Robledo and Seijo 2010). The single wild tetraploid species, *A. monticola*, is very closely related to *A. hypogaea* (Lu and Pickersgill 1993), probably sharing the same origin, and it is considered *A. hypogaea*'s immediate tetraploid ancestor (Seijo et al. 2007; Grabiele et al. 2012). The ploidy barrier between cultivated peanut and most of its wild relatives (with the single exception of *A. monticola* $2n=40$) effectively prevented the introgression of wild genes into cultivated peanut and created a strong genetic bottleneck.

The most frequent of the genome types among the species is the A genome (Fig. 12.1). It is characterised by the presence of a chromosome pair of reduced size and by chromosomes with strongly condensed centromeric bands (Husted 1936; Seijo et al. 2004). The next most frequent genome type is B, which is characterised by the lack of a small chromosome pair and by chromosomes with a much lower degree of centromeric DNA condensation. The genome types F and K were formerly considered B genome species, and their recent classification was based on rDNA loci and the presence in most chromosomes of strongly condensed centromeric bands (Robledo and Seijo 2010). Phylogenies based on DNA sequence data strongly support the validity of these genome divisions (Moretzsohn et al. 2004, 2013; Milla et al. 2005; Tallury et al. 2005; Bravo et al. 2006; Bechara et al. 2010; Friend et al. 2010; Grabiele et al. 2012).

12.3 Characterisation of Wild Species

Peanut wild relatives are considered as sources of agriculturally important traits that can be tapped to improve the cultigen. Although a low number of peanut wild accessions have been used in the breeding programmes, as compared to the high diversity that exists in wild species, extensive phenotypic characterisation of peanut wild relatives has been performed for many traits.

12.3.1 Resistance to Pests and Pathogens

Resistance to pests and pathogens have been identified in several species belonging to the genus *Arachis* (Upadhyaya et al. 2011b). When crosses with the cultivated species are possible, wild species can be used as a source of disease resistance for cultivated peanut, thanks to their broad resistance spectrum and because they are effective against a disease for which variability is not available in the cultivated species. One good example of a source of resistance that fits these criteria is the accession GKP10017 (PI 262141) of the wild species *A. cardenasii* that has been used in the US breeding programmes to transfer resistances for both early and late leaf spot, nematodes and insects into cultivated species. Moreover, Singh and Oswalt (1991) reported accessions of *A. duranensis*, *A. stenosperma*, *A. cardenasii*

and *A. villosa* combining immunity and/or resistance to rust and early or late leaf spot, tomato spotted virus, thrips and/or aphids.

Resistance to disease might have complex inheritance that involves several components including initial infection, lesion size and number, sporulation and defoliation. Recently, Mallikarjuna et al. (2012a) characterised several diploid species, their hybrids and auto- and allotetraploid derivatives. They reported that they combined several components of resistance to late leaf spot. Leal-Bertioli et al. (2009) phenotyped an F_2 population derived from the cross between two wild diploid species *A. duranensis* and *A. stenosperma* for resistance to late leaf spot and expressed the results as percentages of diseased leaf area (DLA). The susceptible parent had an average of 4.53 % DLA, and the resistant parent had 0.15 % DLA. Among the 93 F_2 plants, 73 had lower percentage DLA than the resistant parent, of which 47 had no lesion attesting for important transgressive segregation. Finally, five QTLs of resistance to late leaf spot were detected. Moreover, resistance for foliar diseases was characterised at the microscopic level, and it was found that in *A. stenosperma*, resistance occurs at the pre-penetration level (Leal-Bertioli et al. 2010). The same species also harbours resistance against root-knot nematode, a resistance that is manifest in at least two distinct levels: very few nematodes that penetrate the roots and those that are unable to set up an infection because of a hypersensitive-like response (Proite et al. 2008).

Despite the high level of disease resistance generally found in peanut wild relatives, important variation can exist between accessions belonging to the same species. Singh et al. (1996) conducted a detailed characterisation of 42 accessions belonging to the species *A. duranensis* using principal component analysis and showed that differences in reaction to rust between accessions of the same species explained 15 % of the total variation. Variation between accessions for resistance to pests and diseases has also been reported for nematodes (Sharma et al. 1999), peanut bud necrosis virus (Reddy et al. 2000), late leaf spot and rust (Pande and Rao 2001). These results indicated that clear identification of most resistant wild accessions is needed before their utilisation as source of disease resistance.

12.3.2 Tolerance to Abiotic Stresses

Unlike resistance to pests and diseases that is generally governed by one or a few genes, tolerance to abiotic stress is often polygenic, subject to G×E interactions and thus necessitates accurate phenotyping to better capture the genetic component of trait variation. Field evaluation of peanut wild relatives over several years is difficult because of their differences in generation time (annual to perennial life cycles) and the difficulty of harvesting seeds. Hence, only a limited number of studies have reported the characterisation of peanut wild relatives for response to abiotic stress, and most of them were based on the measurement of morphological and/or physiological traits in greenhouse conditions. Nautiyal et al. (2008) evaluated 38 accessions of 12 *Arachis* species belonging to four sections for thermo-tolerance (heat and cold).

Several morphological and physiological traits, viz., leaf morphology, electrolyte leakage, leaf water potential, specific leaf area (SLA) and leaf chemical constituents, were recorded. Tolerance to heat and cold has been expressed as percentage of leaf relative injury (RI). These authors reported important variation between species and between accessions within a species. One accession of *A. glabrata* (section *Rhizomatosae*) and one accession of *A. paraguariensis* (section *Erectoides*) were identified as heat resistant and cold resistant, respectively. One accession of *A. appressipila* (section *Procumbentes*) was found susceptible to both cold and heat. Moreover, positive correlations were found between RI and SLA both for heat and cold resistance, indicating that genotypes with thicker leaves also have higher tolerance. Upadhyaya et al. (2011a) measured 41 morpho-agronomic traits over 269 accessions from 20 wild *Arachis* species belonging to six sections. A large range of variation was observed for traits related to drought: earliness, SLA and SPAD chlorophyll meter reading (SCMR). Finally, a set of 20 accessions with superior agronomic and drought-related trait combinations were proposed to be used in breeding programmes for introgression of wild favourable alleles in the genetic background of cultivated varieties. Leal-Bertioli et al. (2012) investigated drought-related traits such as leaf morphology, transpiration profile, SCMR, SLA and transpiration rate per leaf area of two wild diploid species (*A. duranensis* and *A. ipaënsis*) and one synthetic tetraploid deriving from the cross between the same diploid species. One interesting result that came from this study is that most drought-related traits such as leaf area, stomata size and transpiration rate were substantially modified when evaluated in a wild tetraploid context attesting for effects of the ploidy level on trait variations. These authors concluded that, for the introgression of drought-related traits from wild species to cultivated species, evaluation of the synthetic tetraploid was likely to be more informative than the evaluation of the diploids. Additionally, it would also be interesting to investigate the effect of genetic background (cultivated versus wild) on the modification of drought-related trait variation.

12.3.3 Evaluation for Nutritional Value

As peanut is an important food and oil crop, improving protein content and oil quality in seeds are important objectives of breeding programmes. The properties of peanut oil are determined by the fatty acid content and particularly the oleic-to-linoleic ratio (O/L). The analysis of the chemical composition of peanut wild species seeds has been reported. The oil and protein content and the fatty acid and sterol composition of the seeds of several wild accessions were studied by Stalker et al. (1989) in sections *Arachis*, *Heteranthae*, *Caulorrhizae* and *Procumbentes* and by Grosso et al. (2000) in sections *Arachis*, *Extranervosae*, *Erectoides* and *Triseminatae*. The highest oil content and O/L ratio were found in species belonging to section *Arachis* (*A. stenosperma* and *A. villosa*, respectively). In both studies, none of the wild species analysed overrode the cultivated species in terms of chemical quality and oil stability. However one can expect that the cultivated species could benefit from

positive alleles from the wild through transgressive segregation. Jiang et al. (2009) evaluated 87 wild *Arachis* accessions and 113 interspecific offspring for traits related to fatty acid composition. These authors reported transgressive segregation in progeny derived from the crosses between *A. stenosperma* and two Chinese cultivated varieties and between *A. glabrata* and one cultivated line. Two progenies involving *A. stenosperma* as wild donor had 12.8 to 29.7 % more oleic acid than their parents. Similarly, four progenies from the cross between *A. glabrata* and one cultivated line had higher content of oleic acid and lower content of palmitic acid than the cultivated line. Moreover, in this study, high content of oleic acid was found in accessions of *A. duranensis* and *A. pusilla*. Wang et al. (2010) evaluated 39 wild species of different sections for oil content, fatty acid composition and D150N functional mutation of the *FAD2A* gene. Significant variability was found among species, but no accession had high oleic/linoleic acid ratio. Finally, Upadhyaya et al. (2011a) evaluated the nutritional value (oil, protein and sugar) of 20 peanut wild accessions among which seven belong to *A. stenosperma*, three each to *A. monticola* and *A. pusilla*, two to *A. kuhlmannii* and one each to *A. villosa*, *A. batizocoi*, *A. duranensis*, *A. dardani* and *A. paraguariensis*. These authors reported for oil content a range of variation (45–55 %) similar to that reported in cultivated varieties.

12.4 Molecular Markers and Maps: The Introgression Toolbox

Plant breeding programmes generally use backcrossing for the introgression of wild genes into elite materials for a specific trait. At each backcross generation, plants with the wild target phenotype introgression are selected, while the background of the cultivated parent is recovered through generations. Without the help of molecular markers, this process results in the introgression of a large portion of the donor genome, which carries undesirable genes associated to the allele of interest (linkage drag), and many backcross generations are necessary to eliminate the deleterious genes. Molecular markers, ordered on a genetic map, provide a tool to monitor the size and distribution of wild introgressions throughout the breeding process. Their availability is a key step in the successful implementation of large-scale introgression programmes.

12.4.1 Molecular Markers

The first markers used in peanut were isozymes and proteins (Grieshammer and Wynne 1990; Krishna and Mitra 1988; Lu and Pickersgill 1993), followed by restriction fragment length polymorphisms—RFLPs (Kochert et al. 1991, 1996; Paik-Ro et al. 1992), random amplified polymorphic DNA—RAPD (Dwivedi et al. 2001; Halward et al. 1991, 1992; Hilu and Stalker 1995; Raina et al. 2001;

Subramanian et al. 2000) and amplified fragment length polymorphism—AFLP (Gimenes et al. 2002; He and Prakash 1997, 2001; Herselman 2003; Milla et al. 2005; Tallury et al. 2005). However, none of these marker systems were very informative in cultivated germplasm. In recent years, microsatellite or simple sequence repeat (SSR) markers have become the assay of choice for genetic studies in *Arachis*, since they are multiallelic, co-dominant, polymorphic, transferable among related species, PCR based and usable in tetraploid genomes. In consequence, efforts have been made by several research groups to develop microsatellite markers for peanut. Up to 15,000 SSR markers have been published to date (Hopkins et al. 1999; Palmieri et al. 2002, 2005; He et al. 2003, 2005; Ferguson et al. 2004; Moretzsohn et al. 2004, 2005, 2009; Bravo et al. 2006; Budiman et al. 2006; Gimenes et al. 2007; Proite et al. 2007; Wang et al. 2007, 2012; Cuc et al. 2008; Gautami et al. 2009; Guo et al. 2009; Liang et al. 2009; Nagy et al. 2010; Song et al. 2010; Yuan et al. 2010; Koilkonda et al. 2012; Macedo et al. 2012; Shirasawa et al. 2012a). The availability of a great number of microsatellite markers has enabled access to the low genetic variation available in cultivated peanut (Barkley et al. 2007; Krishna et al. 2004; Macedo et al. 2012; Tang et al. 2007; Varshney et al. 2009b). Recently, Shirasawa et al. (2012b) developed 535 markers derived from transposon-enriched genomic libraries. These MITE markers showed great potential, as they detected higher polymorphism levels than genomic microsatellite markers. Finally, single-nucleotide polymorphism (SNP) markers constitute the most abundant molecular markers in the genome and can be carried out with higher throughput genotyping methods. SNP markers have been widely used in many plant species. However, they have not been used in peanut so far, as the implementation in polyploid plants is difficult.

12.4.2 Genetic Maps

Linkage maps are particularly useful for the study of the genome structure and organisation and for marker-assisted selection in breeding programmes. Due to the very low genetic variation in cultivated peanut, interspecific populations have first been used for the construction of linkage maps in *Arachis*.

The first published map for *Arachis* was based on RFLP markers and developed using an F_2 population of 87 individuals derived from a cross *A. stenosperma* × *A. cardenasii*, both diploid species with A genome (Halward et al. 1993). One hundred and seventeen loci were mapped into 11 linkage groups covering a total map distance of 1,063 cM. A diploid backcross population derived from the same parents was also used to compute a linkage map (Garcia et al. 2005). One hundred and sixty-seven RAPD and 39 RFLP loci were mapped into 11 linkage groups, spanning 800 cM. The 39 RFLP markers were common to the F_2-based map of Halward et al. (1993) and were used to establish correspondences between both maps. All common markers mapped to the same linkage groups and mostly in the same order.

The first genetic map for the tetraploid genome of *Arachis* was based on a backcross population (BC_1) having the amphidiploid TxAG-6 [*A. batizocoi* ×

(*A. cardenasii* × *A. diogoi*)]^{4x} as donor parent and *A. hypogaea* cv. Florunner as recurrent parent (Burow et al. 2001). A total of 370 RFLP markers were mapped into 23 linkage groups covering 2,210 cM. The pairing of homoeologous linkage groups was consistent with the disomic nature of the allotetraploid peanut.

The first peanut SSR-based map was constructed for an F_2 population derived from a cross of two diploid species with A genome, *A. duranensis* and *A. stenosperma* (Moretzsohn et al. 2005). One hundred and seventy loci were mapped into 11 linkage groups covering 1,231 cM of total map distance. New markers were added to this map, resulting in 369 loci, including 188 microsatellites, 80 anchors and 35 resistance gene analogue (RGA) markers, mapped into ten linkage groups, as expected for diploid species of *Arachis* (Leal-Bertioli et al. 2009). The same authors developed a diploid F_2 population derived from the cross *A. ipaënsis* × *A. magna* to build a map for the B genome of *Arachis* (Moretzsohn et al. 2009). A total of 149 co-dominant markers, mostly microsatellites, were mapped into ten linkage groups spanning a distance of 1,294.4 cM. Fifty-one common markers evidenced the high synteny of the B genome map and the A map and revealed the translocation of chromosomal segments from the group A7 to the group A8.

A synthetic amphidiploid (*A. ipaënsis* × *A. duranensis*) was crossed and backcrossed to *A. hypogaea* cv. Fleur 11, resulting in 88 BC_1F_1 individuals (Fonceka et al. 2009). This population was used to develop an SSR-based linkage map for the tetraploid genome, composed of 298 markers and 21 linkage groups, covering a total distance of 1,843.7 cM. The segregation analysis indicated a disomic inheritance of all loci and chromosome pairing occurring between homologous genome confirming the close relationship between the wild diploids *A. duranensis*, *A. ipaënsis* and the cultivated peanut and highlighted structural rearrangements, such as chromosomal segment inversions and a major translocation event, between the A and B genome species. A comparative analysis of this map with the diploid genome maps of Moretzsohn et al. (2005, 2009) suggested the occurrence of this event prior to the peanut's tetraploidisation.

An intraspecific map was published in 2008, using 142 individuals of a recombinant inbred line (RIL) population derived from a cross between one accession of *A. hypogaea* subsp. *hypogaea* and one accession of the *fastigiata* subspecies (Hong et al. 2008). New markers were added to this map, and two additional maps were constructed based on RIL populations having accessions of the subspecies *fastigiata* as parents (Hong et al. 2010). Of the 901 screened, 132, 109 and 46 SSR markers were mapped on each of the three linkage maps. A reference map was developed, with 175 loci and 22 linkage groups, covering a total distance of 885.4 cM. The marker order was in general collinear to the A genome map of Moretzsohn et al. (2005).

Another intraspecific map for peanut was developed using a RIL population composed of 318 F_8/F_9 plants (Varshney et al. 2009a). Corroborating the known low genetic variation of peanut, only 135 microsatellite markers, of the 1,145 screened, mapped in 22 linkage groups spanning 1,270.5 cM. This map enabled the mapping of QTLs controlling drought tolerance-related traits as well as establishing relationships with diploid A genome of groundnut and model legume genome species.

In 2012, several moderately saturated maps and two proposed reference maps were published. Wang et al. (2012) reported a linkage map, based on an F_2

population of 94 individuals derived from *A. hypogaea* subsp. *hypogaea* × *A. hypogaea* subsp. *fastigiata* cross. The map consisted of 318 loci, mostly SSRs, onto 21 linkage groups covering a total distance of 1,674.4 cM. In this study, resistance gene homolog (RGH)-containing BAC clones were sequenced to develop SSR markers. Two of these markers were mapped into two different linkage groups, anchoring one RGH-BAC contig and one singleton, which can facilitate marker-assisted selection for disease resistance breeding and map-based cloning of resistance genes. Shirasawa et al. (2012a) developed a great number of microsatellite and transposon markers by in silico analysis and published the most saturated individual map for *Arachis* to date. A total of 1,114 markers were mapped into 21 linkage groups covering 2,166.4 cM based on an F_2 population ($n = 94$) derived from an *A. hypogaea* subsp. *hypogaea* × *A. hypogaea* subsp. *fastigiata* cross. The authors also published an intra-subspecific *A. hypogaea* subsp. *hypogaea* × *A. hypogaea* subsp. *hypogaea* map, with 326 markers and 19 linkage groups, spanning 1,332.9 cM.

An integrated map was constructed from two RIL populations (Qin et al. 2012). This map contained 324 markers covering 1,352.1 cM with 21 linkage groups. The translocation event that seems to have occurred between linkage groups A7 and A8 or B7 and B8 was also evident in comparison of this map to previously published diploid and tetraploid maps (Moretzsohn et al. 2005, 2009; Fonceka et al. 2009).

Two reference consensus linkage maps were published very recently for peanut (Gautami et al. 2012; Shirasawa et al. 2013). The first map (Gautami et al. 2012) was based on ten intraspecific RILs and one interspecific backcross population and comprised 895 microsatellite markers. Linkage groups were identified and named by comparisons with the diploid maps previously published for the A and B genomes of *Arachis* (Moretzsohn et al. 2005, 2009; Leal-Bertioli et al. 2009). The reference map was divided into 20 cM long 203 BINs having microsatellite markers with known polymorphism information content (PIC) values. The second map (Shirasawa et al. 2013) was based on three wild-derived RIL populations, integrated with another 13 maps already published and with sequences of other legume species. All this information will be useful for selecting highly polymorphic and uniformly distributed markers for further genetic studies and marker-assisted selection in peanut.

12.5 Achievements

12.5.1 The Different Routes for Interspecific Population Development: How to Access Wild Gene Reservoir in the Context of Ploidy Reproductive Barrier

One of the main constraints to hybridisation between cultivated peanut *A. hypogaea* and its wild relatives is the reproductive barrier caused by the difference in ploidy level. In order to access and use the large genetic diversity available in diploid species in a tetraploid context, one has to turn to bridging strategies allowing the crossability of wild species and the cultivated. Four different routes have been

described by Simpson (2001): the hexaploid pathway, two different diploid/tetraploid pathways and genetic engineering-based methods.

The hexaploid pathway consists in the direct cross of a given diploid species with *A. hypogaea*. The resulting hybrid is triploid and sterile and can be doubled to the hexaploid level after treatment by colchicine. In general, the hexaploid is cytologically unstable, and the tetraploid state needs to be recovered following chromosome loss through successive selfing or backcrossing to *A. hypogaea*.

The first diploid/tetraploid route consists of reconstructing a tetraploid from a two- or a multiple-way cross of different *Arachis* diploid species having different genome types. The hybrid is treated by colchicine to double the chromosome number. The resulting allotetraploid is cross compatible with cultivated peanut and can be used as a parent for an introgression programme. Alternatively, in the second diploid/tetraploid route, a single *Arachis* diploid species representative or hybrids between diploid species having the same genome can be colchicine doubled to form an autotetraploid that is cross compatible with cultivated peanut. Autotetraploids have however been described as weak plants and crossing with *A. hypogaea* reported as difficult (Holbrook and Stalker 2003).

As a fourth strategy, transformation technologies can be used to access genes from species of the tertiary gene pools or from outside the genus.

12.5.2 Wild Introgressions in Peanut: A Historical View

Several long-term programmes have been conducted to introgress valuable genes from wild *Arachis* species into cultivated peanut since the first interspecific hybridisations realised by Krapovickas and Gregory in the 1940s.

Using the hexaploid pathway, Stalker et al. (1979) generated a tetraploid interspecific population from a cross produced earlier by Smartt and Gregory (1967) between a cultivated peanut line (PI 261942-3), collected from Paraguay, and the A genome diploid species *A. cardenasii* (10017 GKP, PI 262141). The population was obtained after five generations of selfing from the colchicine-doubled triploid hybrid that allowed to recover tetraploid individuals with 40 chromosomes. The phenotypic characterisation of this population allowed the identification and selection of several lines for higher yield, resistance to leaf spots (*Cercospora arachidicola* and *Cercosporidium personatum*) as well as resistance to several insects. Using the ten highest yielding families of this population, a recurrent selection programme was conducted (Guok et al. 1986) and resulted in a significant increase in fruit yield and kernel yield components after two cycles of recurrent selection, providing an early evidence that favourable alleles for grain production can be gained from a wild *Arachis* diploid species. From the same population, several hybrid selections were identified as having significantly higher level of resistance to early leaf spot (Stalker 1984) than the most resistant cultivar evaluated at the same time. However, these hybrid derivatives had poor agronomic performance and could not be used directly as improved variety.

With the development of molecular markers, the same group of researchers characterised a set of lines from the same interspecific population with RFLP and RAPD markers (Garcia et al. 1995). Based on an existing RFLP linkage map (Halward et al. 1993), the analysis revealed the distribution of introgressed segments from *A. cardenasii* in the cultivated genetic background attesting of recombination events between the diploid genome and both genomes of the cultivated (88 % of the *A. cardenasii* introgression events were located in the A genome and 12 % were located in the B genome). One of the lines, identified as resistant to nematode (*Meloidogyne arenaria*), was crossed again to the cultivated parent to generate a segregating population from which two linked dominant resistance genes could be identified and designated as *Mae*, a dominant gene restricting egg number, and *Mag*, a dominant gene restricting galling. Using bulked segregant analysis (BSA), one RAPD marker was linked at 10 and 14 cM from *Mag* and *Mae*, respectively (Garcia et al. 1996). Two root-knot nematode-resistant varieties have been released following this research (Stalker et al. 2002a). In addition, late leaf spot resistance lines deriving from the same population were also registered (Stalker and Beute 1993; Stalker et al. 2002b) as well as insect resistant lines (Stalker and Lynch 2002).

More recently, the variety GPBD4 has been developed through pedigree selection from the cross between KRG1, an early maturing line from Argentina, and ICGV 86855 (Gowda et al. 2002), also referred to as CS16 (Vishnuvardhan et al. 2011), an interspecific derivative from *A. cardenasii*. GPBD4 is resistant to late leaf spot and rust and has spread over large area in the state of Karnataka in south of India (Gowda et al. 2002). A QTL mapping study involving a RIL population from the cross between GPBD4 and TAG24 allowed the identification of late leaf spot and rust resistance QTLs that could be related to early introgressions from *A. cardenasii* into the cultivated genome (Khedikar et al. 2010). Another case of utilisation of *A. cardenasii* was the development of a foliar disease-resistant variety using the hexaploid pathway from a primary cross between the variety CO1 and *A. cardenasii*. After three successive backcrosses with the cultivated parent and four generations of selfing, the variety VG9514 was selected (Varman 1999), and it showed good resistance to rust and late leaf spot. The rust resistance QTL identified by Khedikar et al. (2010) from GPBD4 was confirmed in a similar RIL population involving VG9514 and TAG24 as parents (Mondal et al. 2012).

Following the work with *A. cardenasii*, another landmark in the use of wild species occurred with the use of the tetraploid route. Simpson et al. (1993) created the first amphidiploid from a three-way cross between *A. cardenasii* and *A. diogoi* on the A genome side and *A. batizocoi* on the B genome side. The AB sterile hybrid was treated with colchicine to produce TxAG-6, a fertile amphidiploid that has been at the root of major genetic studies and breeding applications. The first tetraploid RFLP-based genetic map was constructed from an interspecific BC_1 population involving TxAG-6 as donor parent into the cultivated background of Florunner (Burow et al. 2001). The genetic map obtained was the first nearly saturated tetraploid map and allowed the genome-wide analysis of the transmission of chromatin between wild and cultivated species attesting of a similar recombination pattern as chromosome pairing reported for *A. hypogaea*. The RFLP nature of the markers

used in this study also made it possible to analyse the synteny conservation and colinearity between the two subgenomes of *A. hypogaea* showing a globally high level of conservation and some chromosomal rearrangements. TxAG-6 and TxAG-7, a backcross derivative (BC_1) of TxAG-6 with the variety Florunner, were released for their breeding potential for resistance to root-knot nematode and leaf spot (Simpson et al. 1993). TxAG-7 was further used as parent of a backcross population (BC_4F_2) from which genetic markers linked to root-knot nematode have been identified by bulk segregant analysis. This single gene, identified in *A. cardenasii* and transferred to peanut through TxAG-6, is currently the only dominant root-knot nematode resistance gene deployed in modern cultivars (Holbrook et al. 2008). By comparative genetic mapping in diploid and tetraploid peanut populations, this gene, called *Rma*, was found to have been introduced in a chromosome segment spanning one-third to one-half of chromosome 9A (Nagy et al. 2010). In the latter study, numerous codominant markers were identified for finer mapping of *Rma* and for marker-assisted selection for nematode resistance, by using two tetraploid RIL populations of *A. hypogaea* and an intraspecific F_2 diploid population from a cross between two *A. duranensis* accessions. Initially, two varieties were released from backcross derivatives of TxAG6 with Florunner: COAN and NemaTAM (Simpson and Starr 2001; Simpson et al. 2003). NemaTAM was almost immune to root-knot nematode but sensitive to tomato spotted wilt tospovirus (TSWV). Further improved varieties were developed starting either from NemaTAM or COAN and using either conventional methods or marker-assisted selection to develop varieties combining resistance to nematode and TSWV, like Tifguard (Timper et al. 2008) or Tifguard high O/L (Holbrook et al. 2011).

Another route, involving in vitro techniques, has also been described that allowed to tap wild species from tertiary gene pool of *Arachis* genus. Hybrids have been obtained between *A. hypogaea* and two diploid wild species from section *Procumbentes*, *A. chiquitana* and *A. kretschmeri* (Mallikarjuna and Hoisington 2009; Mallikarjuna and Tandra 2006). *Arachis glabrata* from section *Rhizomatosae* has also been successfully crossed with *A. hypogaea* (Mallikarjuna and Sastri 2002). The method includes embryo rescue from immature pods resulting from the interspecific cross between *A. hypogaea* and the diploid wild. To recover tetraploid state the authors benefited from numerically unreduced gametes or 2*n* pollen that are produced at low frequency from F_1 hybrids (Mallikarjuna and Tandra 2006). The same authors have also used the tetraploid route to produce a collection of 17 new allotetraploids and autotetraploids between different species of the secondary gene pool (Mallikarjuna et al. 2011). The different diploid species involved were *A. batizocoi*, *A. cardenasii*, *A. diogoi*, *A. duranensis*, *A. hoehnei*, *A. ipaënsis*, *A. kempff-mercadoi*, *A. magna*, *A. stenosperma* and *A. valida*. One allotetraploid (ISATGR 1212) and its reciprocal form (ISATGR-40A) had the same genomic composition of *A. hypogaea* originating from the cross of *A. duranensis* with *A. ipaënsis*. This collection of synthetics representing a wide coverage of diversity of the secondary gene pool of *Arachis* are a valuable resource for introgression of positive wild alleles into cultivated gene pool, and the crossability with cultivated peanut has been analysed for five of them (Mallikarjuna et al. 2012b). The use of these synthetics as

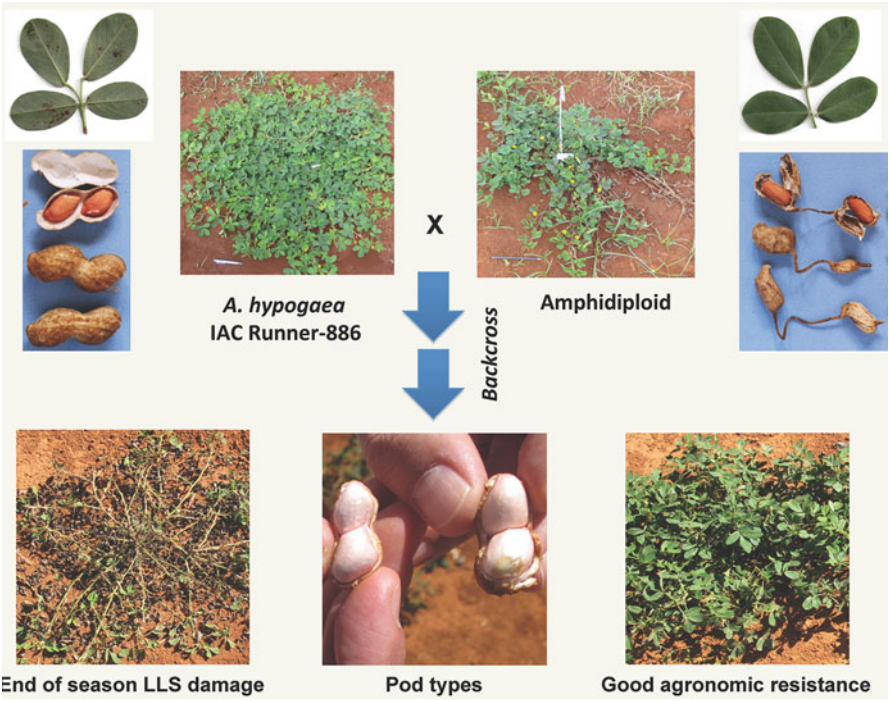

Fig. 12.2 Introgression of resistance for late leaf spot into the cultivated variety Runner IAC 886. After one backcross and field selection of backcross, lines combining resistance to LLS and good agronomic performance were selected

progenitors to conduct introgression programmes in cultivated peanut is in progress in breeding programmes in Senegal and in India.

Also using the tetraploid route, a synthetic amphidiploid has been developed in Brazil from the proposed ancestors of cultivated peanut (*A. ipaënsis* and *A. duranensis*) (Fávero et al. 2006). This amphidiploid donor has been crossed with Runner IAC 886 (a selection of Florunner), and 12 lines that combine agronomically adapted phenotype with resistance to late leaf spot have been selected using a combination of genotyping and phenotyping (Fig. 12.2) (Leal-Bertioli et al. 2010; Leal-Bertioli SCM, Moretzsohn MC, Guimaraes PM, Godoy I and Bertioli DJ unpublished results).

An introgression programme has been conducted using the same amphidiploid and Fleur 11, a popular variety from Senegal, as cultivated recipient. The whole process involved the construction of an interspecific SSR genetic map at the BC_1F_1 generation (Fonceka et al. 2009), followed by a large marker-assisted backcross scheme to monitor wild introgression distribution in the genome of the cultivated parent at each generation. The programme was conducted up to the BC_4F_3 generation to produce a set of chromosome segment substitution lines (CSSLs) that globally incorporate the whole genome of the wild ancestors as overlapping segments

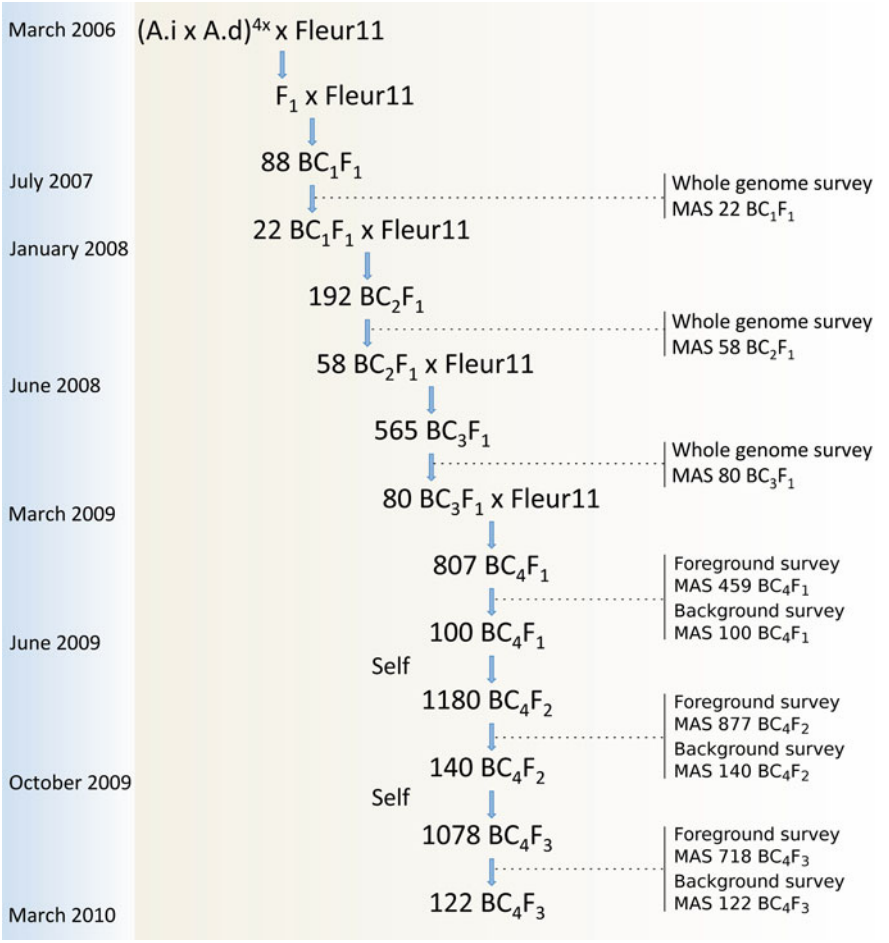

Fig. 12.3 Breeding scheme followed for the development of the CSSL population (Fonceka et al. 2012b)

introgressed in the recipient cultivar. The whole programme that represents seven generations was conducted over a period of 4 years (Fig. 12.3) thanks to off-season generations and the ability to successfully genotype progenies as part of the breeding process during the short time frame between seedling and flowering.

As part of this introgression programme, an advanced backcross (AB-QTL) population was derived from the plants that were not selected to be advanced to CSSLs at the BC_2F_1 generation (Fonceka et al. 2012a). The population was composed of a mix of BC_3F_1 and BC_2F_2 individuals that were allowed to self-pollinate to produce BC_3F_2 and BC_2F_3 families used for the phenotyping and QTL detection. Several traits concerning days to flowering, plant architecture, pod and seed morphology and yield components were analysed in this population leading to the identification of 95 QTLs in two different water regimes. As a first conclusion, it could be shown

that wild alleles contributed positive variation to many valuable agronomic traits such as flowering precocity, seed and pod number per plant, length and size as well as pod maturity. About half of the positive QTL effects were associated to the allele of the amphidiploid parent. In some cases, these QTLs, such as QTLs for seed length on chromosome a09, were associated to undesirable morphological traits like pod constriction or pod beak, probably requiring further backcrosses to reduce linkage drag. However, in several cases favourable wild QTLs had no detrimental association and could be directly used to improve the cultivated variety: QTLs for pod number and weight on chromosome a01, QTLs for seed number, total biomass and stress tolerance indices on chromosome a05 and QTLs for seed diameter on chromosome b06. Moreover, the comparison of QTLs obtained under well-watered and water-limited conditions revealed that QTLs for stress tolerance indices for pod and seed numbers with favourable alleles attributed to the wild parents could be involved in the trade-off between maintaining large-sized seed and producing more seeds under water stress. In addition, QTL clusters related to domestication syndrome, i.e. involved in plant and pod morphology as well as pod and seed size, were also mapped in the same study. QTLs that greatly affected pod and seed size appeared to be clustered in three genomic regions while those affecting the plant and pod morphology were dispersed across the genome. It was proposed from these findings that the main focus of human selection at the incipient stage of domestication could have been concentrated on pod and seed size, given the primitive growth habits and constriction depths that still exist in peanut cultivated species (Fonceka et al. 2012a).

The final CSSL population (Fonceka et al. 2012b) was composed of 122 lines offering a wide coverage of the peanut genome especially in the context of the large peanut genome size (c. 2,800 Mb/1C and 20 linkage groups) with target wild chromosome segments of 39.2 cM on average. Most of the CSSLs (62 %) contained a single wild fragment in a homogeneous cultivated genetic background (Fig. 12.4). For the lines that contained more than one fragment, additional backcrossing efforts are ongoing for deriving lines harbouring a unique wild chromosome segment. Using simple high-heritability traits like plant growth habit or pod constriction as a proof of concept, the value of the CSSL population could be illustrated. For example, an introgression line harbouring a single wild donor fragment corresponding to the location of a QTL for pod constriction identified in the AB-QTL population showed deeply constricted pods as compared to the moderate constriction observed with the cultivated parent, confirming the QTL previously identified. Similarly, two lines harbouring single overlapping donor fragments in the region of a QTL for plant growth habit showed contrasting phenotypes, allowing to confirm and refine the position of this QTL (Fig. 12.5).

12.6 Conclusion and Further Prospects

As described in the previous sections, wild introgressions in peanut have now been carried out for many years, particularly because of the specific interest this approach represents for the improvement of this crop. However, in spite of those important

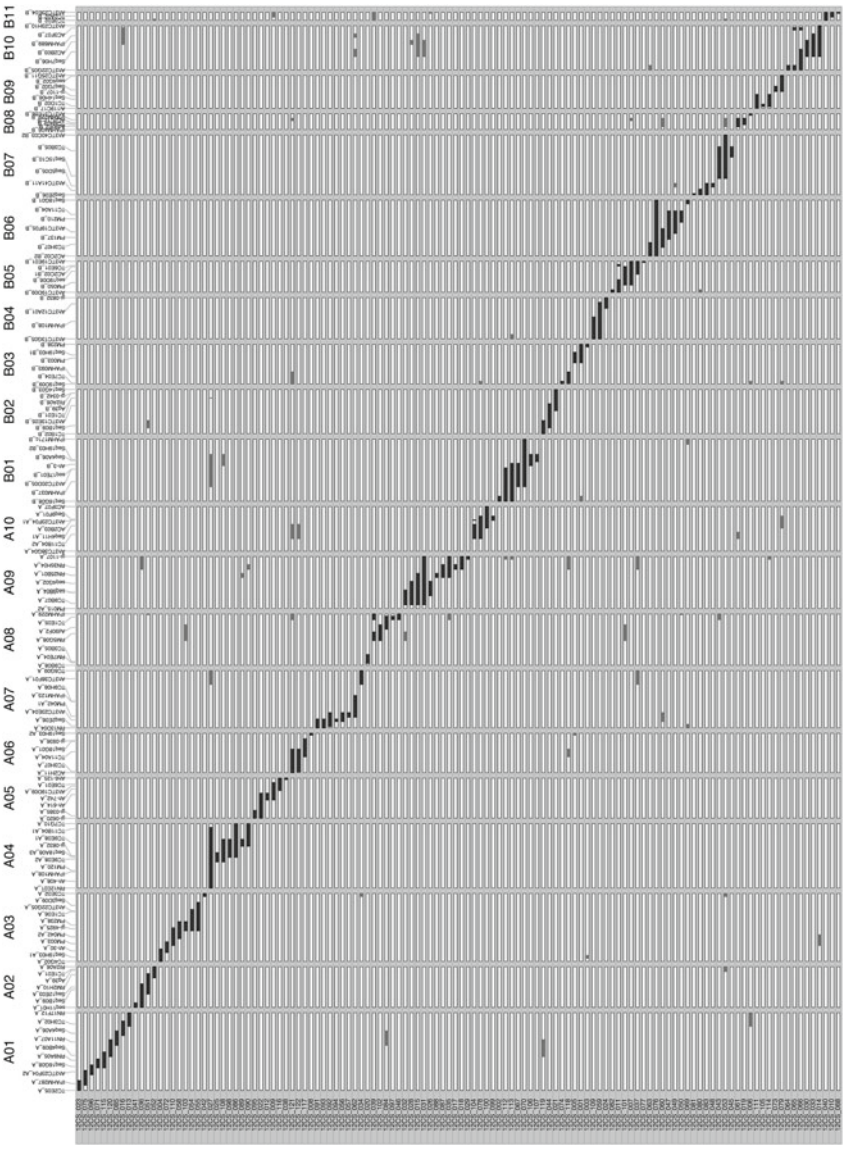

Fig. 12.4 Graphical genotypes of the 122 CSSLs (reproduced from Fonceka et al. 2012b)

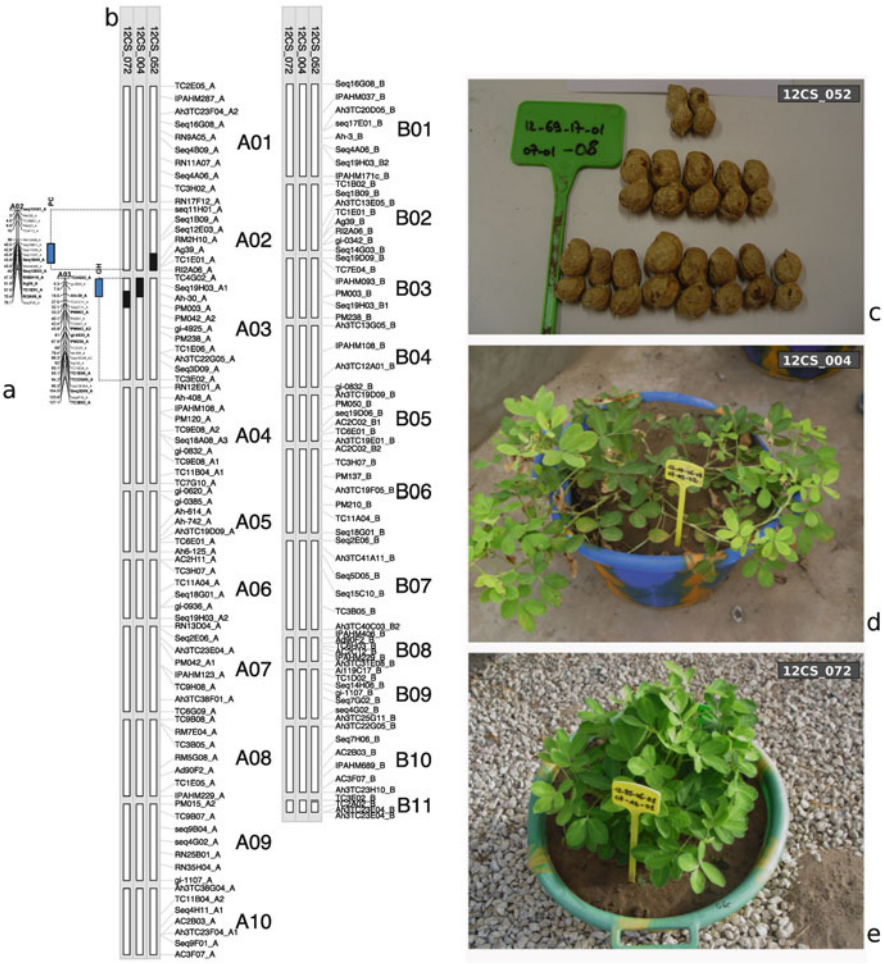

Fig. 12.5 Relation between introgression and phenotype for pod constriction and plant growth habit for three CSSL lines. (**a**) QTLs detected in AB-QTL population for pod constriction (PC) on linkage group a02 and for plant growth habit (GH) on linkage group a03. (**b**) Graphical genotype of three CSSL lines corresponding to the same QTLs. (**c**) Phenotype of the CSSL line 12CS_052 for pod constriction. (**d**) Phenotype of the CSSL line 12CS_004 for plant growth habit. (**e**) Phenotype of the CSSL line 12CS_072 for plant growth habit

achievements, and mainly due to the limitations of the plant itself in terms of crossability, multiplication rate, and, until recently, lack of appropriate molecular tools, the extent of utilisation of the useful allele reservoir of the wild species and its impact on peanut breeding have been limited. For now, successful introgression of wild genes into cultivated peanut concerns few wild species. *Arachis cardenasii* has probably been one of the most used sources of useful genes to date even if crosses involving other species have also been used. The recent use of the two most probable ancestors of peanut *A. duranensis* and *A. ipaënsis* in a systematic

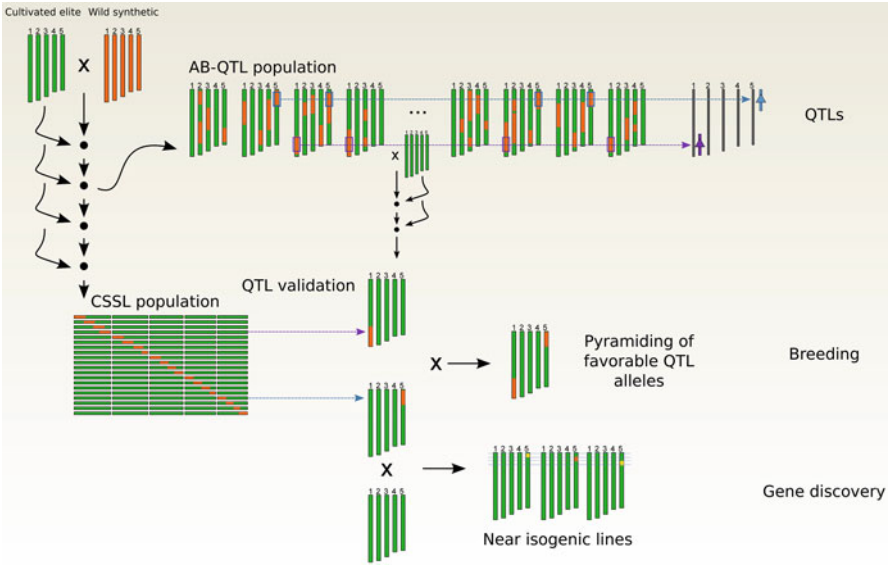

Fig. 12.6 A general strategy for harnessing the potential of peanut wild relatives using AB-QTL and CSSL populations

introgression programme opens the way for extensive and detailed characterisation of genetic determinism and wild alleles' effects on a wide range of traits. However this resource only involves two representatives of two wild species, and the potential in generalising introgression programmes to other accessions of the same species and to other species of the secondary gene pool is immense.

A general strategy to harness this potential is proposed in Fig. 12.6. One of the major lessons learned through the utilisation of wild species in crop improvement is that cryptic valuable alleles can be found in wilds that can be identified and characterised only once it is incorporated in a cultivated genetic background. As proposed by Tanksley and Nelson (1996), advanced backcross QTL analysis has the potential to simultaneously identify wild QTL alleles while delivering genetic material directly applicable to breeding. One extension of this approach is to construct a CSSL library through more backcross generations and a higher control of introgression sizes and distribution. However, this last approach requires a large investment in terms of crossing and genotyping and cannot be generalised to a wide range of wild donors. Nevertheless, single-fragment introgression lines can be derived from AB-QTL populations on a QTL-by-QTL basis. Such lines, once the wild effect has been validated and characterised, can be crossed to accumulate favourable alleles for different traits toward variety release. They can also be used for deriving near-isogenic lines through further backcrossing providing experimental material for map-based gene cloning. The implementation of this strategy requires a close integration of genotyping in the breeding process, which can now be widely achieved, thanks to the development of molecular markers and genetic maps in peanut and the availability of genotyping platforms offering fast and reliable genotyping services.

Such a large-scale generalised introgression programme would require a concerted effort of the peanut international community. Taking into account the number of wild accessions available in gene banks, a rational sampling of target donors would have to be achieved, regarding peanut breeding objectives, based on the characterisation knowledge base that already exists on wild diseases resistance, ecological adaptation, fertility and crossability. A concerted strategy would also be required on the choice of cultivated backgrounds for introgression. A first option (the one that has been used in tomato) would be to use a common cultivated background allowing the direct comparison of the effects of introgressions from different species and the optimisation of the introgression effort among the community. However, the disadvantage of this option resides in the fact that a common cultivated background may not be adapted to some of the target environments compromising the potential of direct breeding application of introgressed material. As an alternative, the choice of specific targeted elite cultivars by improving wild crosses seems to offer a better tradeoff between breeding opportunities and genetic analysis.

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