Chapter 17
Genomics of Peanut, a Major Source of Oil and Protein

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Abstract Peanut, as a source of oil and protein, is the second-most important grain legume cultivated. The perceived lack of molecular variation in the cultivated species had, until recently, resulted in a focus on characterization and mapping of wild species and on transformation of peanut with genes for improved disease resistance. With development of simple sequence repeats and potentially single nucleotide polymorphism-based markers and improved minicore collections, the focus is shifting towards the molecular characterization of the cultivated species. The development of large-inset libraries, expressed sequence tags, genomic clone libraries, characterized mutant collections, and bioinformatics is expected to advance peanut genomics.

17.1 Introduction

Peanut, or groundnut, is a member of the legume family (Leguminosae or Fabaceae). Plants of the cultivated peanut species, *Arachis hypogaea* L., are annuals, either erect (up to 60 cm tall) or prostrate (usually under 30 cm tall). Inflorescences consist of approximately three perfect flowers that usually appear several days apart; the inflorescences occur in the axils of foliage leaves. Peanut differs from most angiosperms in that the fertilized ovary is carried on a peg (gynophore) into the ground, where pod development begins and the fruits are produced. Depending on genotype and environment, fruits mature 2.5 to 6 months after sowing.

Considerable morphological diversity exists among wild peanut species. For example, although many species produce flowers similarly to the cultigen, some

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produce subterranean flowers and some produce rhizomes in addition to flowers. Few wild species are raised commercially, but some are grown for forage. Wild species may possess valuable alleles apparently lacking in the cultigen.

17.1.1 Economic, Agronomic, and Societal Importance of Peanut

Cultivated peanut is the second-most important grain legume crop worldwide after soybean, with 33 million tons of seed produced in 2003/04 (USDA-FAS 2006). Countries producing more than 1 million tons of seed were China, India, the United States, Nigeria, and Indonesia.

Peanut is important for both its versatility and value. The seed typically contains 36% to 54% oil, 16% to 36% protein, and 10% to 20 carbohydrates (Knauft and Ozias-Akins 1995) as well as high amounts of P, Mg, riboflavin, niacin, folic acid, and vitamin E (The Peanut Institute 2006). Worldwide, the largest use is for oil, with the meal being used as a high-protein dietary supplement for human and animal consumption. In the U.S. and some other countries, the peanut seed is used primarily for food, the seed being roasted or boiled and eaten whole or as part of confections, or ground into peanut butter. Peanut fixes nitrogen symbiotically, requires little fertilizer, and improves the quality of the soil in rotation with other crops. Peanut hay may be used for fodder, and the shells used for fuel or livestock feed.

Peanut cultivation also has significant social consequences. The high protein and energy contents make peanut valuable as a subsistence crop in some countries, and the demand for peanut oil allows peanut to be sold as a cash crop. In some countries, peanut is a significant source of income for women, with significant participation in farming, processing, and sales (Balikrishnan et al. 1998).

17.1.2 Geographic Origin of Peanut

The center of origin of *Arachis* is South America, with wild species having been collected in Brazil, Bolivia, Paraguay, Argentina, and Uruguay (Krapovickas and Gregory 1994; Singh and Simpson 1994; Jarvis et al. 2003). The nine sections within the genus comprise 79 defined wild and one cultivated species (Valls and Simpson 2005). Section Arachis has the widest geographical distribution, with 31 wild species collected in five countries.

The cultigen is considered to have originated in northern Argentina or eastern Bolivia, although an origin in Western Peru has been suggested (Simpson et al. 2001). The archaeological record indicates that peanut was domesticated by indigenous peoples at least 3,500 years ago (Singh and Simpson 1994); older peanut specimens consisted only of wild species. At the time of the explorations by the Spanish and Portuguese in the 16th century, peanut was cultivated in many parts of South America, as well as in the Caribbean and Mexico. As a result of explorations, peanut cultivation spread quickly from the Americas to Africa and Asia.

The cultivated species is divided into two subspecies. Subspecies *hypogaea* is characterized by a spreading growth habit, alternating vegetative and reproductive nodes, lack of flowers on the mainstem, medium-to-large seeds, medium-to-late maturity, and the subspecies include botanical varieties *hypogaea* (Virginia and runner market types) and the less-frequently cultivated *hirsuta*. The *fastigiata* subspecies is typified by erect growth habit, sequential reproductive nodes, flowers on the mainstem, small seeds, early maturity, and the subspecies include botanical varieties *fastigiata* (Valencia), *vulgaris* (Spanish), *peruviana*, and *aequatoriana*. The latter two are not cultivated widely.

17.1.3 Peanut as an Experimental Organism

Most genetic research on peanut is focused on varietal improvement. Major research emphases are resistance to biotic and abiotic stresses and edible seed quality. Peanut is also an excellent system for study of several fundamental biological processes.

Numerous biotic stresses cause significant yield losses (Porter et al. 1990). Early (*Cercospora arachidicola* Hori) and late (*Cercosporidium personatum* (Berk. & Curt.) Deighton) leafspot and rust (*Puccinia arachidis* Speg.) cause substantial losses in Africa and Asia; chemical control is available in the United States but is expensive. Sclerotonia blight (*Sclerotinia minor* Jagger) is a major problem in U.S. areas with cool autumns. Nematodes, especially *Meloidogyne arenaria* (Neal) Chitwood and *M. javanica* (Treub) Chitwood, cause significant losses worldwide. Groundnut rosette and tomato spotted wilt viruses cause major losses where insect vectors are present.

Abiotic stress is a growing concern for peanut cultivation. Many production areas are in semiarid environments or have unreliable rainfall, and global climate changes and growing demand for fresh water pose major challenges. Physiological adaptation and selection for drought tolerance have been studied by many researchers (Cruickshank et al. 2003; Reddy et al. 2003). Contamination of peanut under drought stress by *Aspergillus* spp. produces aflatoxin mycotoxins, which can cause liver cancer and suppress immune response.

Quality issues are also important. The high-oleic varieties developed recently in the United States have monounsaturated fatty acid contents similar to olive oil, improved oxidative stability, and beneficial effects on coronary health (O'Byrne et al. 1997). However, certain seed proteins are associated with allergic response in sensitive individuals, and may cause hypersensitive, potentially fatal, reactions in some people.

Peanut has several important characteristics for basic scientific research. The gravitropic development of peg and pod is present in few other species. Subterranean flower development in some wild species contrasts with the cultivated species (Krapovickas and Gregory 1994). The ability to perform symbiotic nitrogen fixation makes peanut an ideal crop for cross-species comparison. The presence of diploid and tetraploid species can also allow for the study of ploidy on gene function,

expression, and crop evolution. It is possible to obtain field production data at many locations, allowing analysis of gene × environment interaction.

Study of peanut genomics has been limited by biological constraints, and many basic tools of genomics have yet to be developed (National Peanut Genome Initiative 2005; Gepts et al. 2005). The peanut genome is large, making insertional mutagenesis and whole-genome sequencing expensive using current technology, and requiring large genomic libraries for physical mapping and positional cloning. The reproductive isolation of *A. hypogaea* from wild species and the limited genetic base of the former made marker analysis difficult until recently. Regeneration of peanut from tissue culture has been characterized by long regeneration times and relative inefficiency. Concern over consumer acceptance and regulatory costs has prevented release of transgenic varieties to date.

17.2 Cytogenetics, Markers, and Species Identification

Some of the first uses of molecular markers have been related to classification of wild species and in identification of the likely diploid ancestors of the cultivated peanut. This origin is of special interest because it could suggest mechanisms for transferring useful wild species alleles to the cultigen.

17.2.1 Cytology and the Origin of Cultivated Peanut

Arachis hypogaea is a tetraploid (2n=4x=40) (Husted 1936), and there is only one other known tetraploid species, *A. monticola*, in section Arachis. The remaining species of section Arachis are diploid and are grouped into three genomes (A, B, and D) each having 20 chromosomes, with the exception of three species with 18 chromosomes (Krapovickas and Gregory 1994; see Valls and Simpson 2005). Hybridization between the cultigen and section Arachis diploids is possible, but no evidence has been found that this has contributed to ongoing gene flow into the cultigen in nature. Cultivated peanut is considered to be an AB tetraploid, arising from hybridization between A and B diploid species (Smartt et al. 1978).

To date, 20 A-genome diploid species have been described (Krapovickas and Gregory 1994); among these are perennials *A. cardenasii, A. diogoi, A. helodes, A. villosa*, and *A. correntina,* and annuals *A. duranensis* and *A. stenosperma*. Based on cytological evidence and cross-hybridization data, *A. cardenasii* was considered originally to be the most-probable A-genome ancestor of *A. hypogaea* (Smartt et al. 1978).

Until recently, only one annual B-genome species had been identified (Smartt et al. 1978); the B genome is always associated with the absence of a specific small pair of A chromosomes (Fernández and Krapovickas 1994). *A. batizocoi* was first proposed as the B genome donor to the cultigen (Smartt et al. 1978). However, cytological measurements by Stalker and Dalmacio (1986) discounted *A. batizocoi* as B-genome donor.

17.2.2 Markers and Insights into the Origin of the Cultivated Peanut

Lack of marker polymorphism in the cultigen using restriction fragment-length polymorphism (RFLP) and random amplified polymorphic (RAPD) markers (Kochert et al. 1991; Halward et al. 1991) contributed to the hypothesis that all varieties and botanical types of *A. hypogaea* share common diploid progenitors (Kochert et al. 1996). RFLP analysis determined that *A. duranensis* had much greater similarity to *A. hypogaea* than did *A. cardenasii* (Kochert et al. 1991, 1996), leading to the conclusion that *A. duranensis* is the most likely A-genome ancestor. However, subsequent marker analyses have also proposed *A. villosa* (Raina and Mukai 1999), *A. helodes*, and *A. simpsonii* (Milla et al. 2005) as potential A-genome donors.

Molecular marker data from Kochert et al. (1991, 1996) supported *A. ipaënsis* instead of *A. batizocoi* as B genome donor. Fluorescent in situ hybridization analysis using rDNA as labeled probe also suggested *A. ipaënsis* as the donor (Raina and Mukai 1999; Seijo et al. 2004.) Only recently has it been possible to make hybrids between *A. ipaënsis* and *A. hypogaea* (Fávero et al. 2006). Additional work has confirmed the existence of up to 10 B-genome diploids (Krapovickas and Gregory 1994; Valls and Simpson 2005; Milla et al. 2005). This provides additional potential introgression routes into the cultigen.

17.3 Genetic Mapping and Tagging

Development of genetic linkage maps is a common method of studying the structure and organization of the genome of a species. Linkage maps also are needed for mapping of traits and marker-assisted selection, anchoring physical maps from large-insert libraries and positional cloning of important genes, comparisons of synteny within and across species, and ordered genome sequencing.

17.3.1 Markers and Genetic Linkage Mapping

In *Arachis*, five major linkage maps have been reported to date, all involving wild species because of limited molecular variability in *A. hypogaea* (Kochert et al. 1991; Halward et al. 1991). The first genetic linkage map was developed using an F_2 population of a cross between A-genome diploids *A. stenosperma* and *A. cardenasii*. The 117 mapped markers were distributed among 11 linkage groups over 1,063 cM (Halward et al. 1993). A second map based on these parents used a BC_1 population, and added 167 RAPD markers to a skeleton of 39 RFLP markers from the first map (Garcia et al. 2005). The largest map of peanut to date, and the only one to involve a parent from *A. hypogaea,* was constructed from a tetraploid cross of the cultivar Florunner × the synthetic amphidiploid TxAG-6 {*A. batizocoi* × [*A. cardenasii* ×

A. diogoi]}^{4x}. A total of 370 RFLP loci were mapped onto 23 linkage groups, for a map distance of 2,210 cM (Burow et al. 2001). The map was characterized by pairing of homoeologous linkage groups, consistent with a disomic nature of the cultigen.

The first microsatellite-based map was based on an F_2 population from a cross between A genome diploids *A. duranensis* and *A. stenosperma*. The published map had 170 microsatellite markers on 11 linkage groups covering 1,231 cM; further work increased the number of markers to 182 and created the expected 10 linkage groups. To create a map of the B genome, a diploid F_2 population was made by crossing *A. ipaënsis* and *A. magna*. This map has 130 mapped microsatellites and 10 linkage groups (Gobbi et al. 2006). The same marker set was used for both maps, and the relationships are mostly co-linear, in agreement with Burow et al. (2001).

The development of simple sequence repeats (SSRs) may allow mapping crosses involving two cultivated parents. The first characterization of peanut SSRs was made by Hopkins et al. (1999); and several papers have reported the majority of the additional markers (Ferguson et al. 2004b; He et al. 2005; Moretzsohn et al. 2005). This totals 702 markers, about 223 polymorphic for cultivated peanut. Approximately 100 additional SSR primers have been developed from genomic libraries (Umesh Reddy, personal communication) and approx. 2,000 have been developed from screening GenBank and unpublished expressed sequence tags (ESTs) (see Luo et al. 2005a). Short motif repeat number (5–15) dinucleotide repeats present in about 3–4% of ESTs are polymorphic among wild species, however, for cultivated peanut most of the polymorphisms have been found only with longer (15 or more) dinucleotide repeats (Moretzsohn et al. 2005). For most populations involving wild species, this is enough markers for map construction, and the increasing number of SSRs expected from sequencing efforts may make mapping cultivated × cultivated crosses possible soon.

Development of linkage maps from cultivated parents has proved difficult. A partial linkage map was constructed using an F_2 population (Herselman et al. 2004). Five linkage groups with 11 markers spanning 139.4 cM of the genome were reported. Reports of a map of cultivated peanut have been made by He et al. (1999) using AFLP markers; this has been extended by addition of AFLP and SSR markers (He, personal communication).

17.3.2 Placing Arachis *in a Unified Genetic Map of the Papilionoids*

Conservation of gene order is a major finding of genomics, with grasses and brassicas being outstanding examples. Comparative alignment has shown that major genes and quantitative trait loci are often conserved between different crops, providing a powerful tool to generate candidate genes, facilitating identification and cloning of genes that determine key traits. For legumes, a unified genetic map is just starting to be developed (Choi et al. 2004), but to date, peanut has been omitted from this effort.

Development of anchor markers for comparison between genomes is the limiting step in the construction of comparative genetic maps. A database of 459

candidate intron-based legume anchor primers pairs has been published (Fredslund et al. 2006). Using a subset of these primer pairs, 66 size polymorphic, cleaved amplified polymorphisms (CAPs) or derived CAPs (dCAPs) anchor markers have been developed on the A genome mapping population (Hougaard et al. 2005). This is allowing the first comparisons of the *Arachis, Lotus,* and *Medicago* genomes. Although analysis is still underway, some clear blocks of macrosynteny are apparent, for instance between *Arachis* LG6 and one end of *L. japonicus* LG1.

17.3.3 Markers and Phenotypic Analysis

The development of trait maps is a useful approach for evaluating the inheritance and feasibility of accelerating gains from selection. One important objective of gene tagging is marker-assisted selection (MAS). MAS is especially useful for traits which are difficult to measure, have low heritability, or are controlled by a few quantitative trait loci (QTLs) with large phenotypic variance.

A small but growing number of markers have been reported in peanut, almost all for biotic stress resistance. The first markers developed were for resistance to root-knot nematode (*Meloidogyne arenaria*). Root-knot nematode resistance was introduced into *A. hypogaea* from *A. cardenasii* (Garcia et al. 1996). RAPD and sequence characterized amplified region (SCAR) markers were identified for genes for reduced galling and egg number. Three RAPD markers were associated with nematode resistance in several backcross breeding populations derived from the interspecific hybrid TxAG-6, [*A. batizocoi* × (*A. cardenasii* × *A. diogoi*)]^{4x} (Burow et al. 1996). RFLP markers were used in breaking the linkage between resistance and low yield in development of the nematode-resistant variety NemaTAM (Church et al. 2000; Simpson et al. 2003).

Markers for additional traits have been developed also. Stalker and Mozingo (2001) identified RAPD markers explaining up to 35% of phenotypic variation for early and late spot resistance in an interspecific peanut population. Milla et al. (2005) reported AFLP-based markers for *A. cardenasii*-derived resistance to aflatoxin contamination, and a PCR-based marker for resistance to *Sclerotinia minor* have been reported by Chenault and Maas (2005). An AFLP marker explaining 76.1% of phenotypic variation for aphid resistance was identified in a cultivated cross (Herselman et al. 2004). Finally, SNP-based markers for high-oleic trait in peanut have been developed (Patel et al. 2004) and are being used to score a segregating F_2 population (Lopez and Burow 2004).

17.3.4 Defining Regions of the Genome that Control Disease Resistance

An important success of genomics has been the identification and mapping of entire classes of resistance genes. Several dozen such resistance genes have been described in the last decade (see Hammond-Kosack and Parker 2003). Many include the

NB-ARC domain, which is thought to act in signal transduction pathways, and which is commonly referred to as nucleotide binding site (NBS) domain genes.

Using degenerate primers that were designed using motifs conserved in these sequences, resistance gene analogs (RGAs) have been identified in peanut. Seventy-eight NBS-encoding regions were characterized (Bertioli et al. 2003). Of nine markers, four were mapped by RFLP and RGA-display on the A genome mapping population described by Moretzsohn et al. (2005) and Leal-Bertioli (unpublished results). An additional 234 sequences were identified and mapped using overgos onto 250 non-redundant BAC clones (Yüksel 2005).

17.4 Large-Insert Libraries and Physical Mapping

Genome-wide physical maps are important tools for ordered genome sequencing, targeted marker development, and positional cloning. Several technologies exist for making large-insert libraries, but bacterial artificial chromosome (BAC) libraries are currently the most useful method.

The first large-insert library for peanut was developed by Yüksel and Paterson (2005) from a cultivated component line of the variety 'Florunner'. The *Hind*III BAC library contained 182,784 clones, with an average insert size of 104 kb, giving an estimated 6.5× genome coverage. Potential problems in physical mapping are ambiguities associated with duplicated segments on homoeologous chromosomes and difficulties in anchoring contigs to duplicated genetic markers. To investigate the practicality of physical mapping in peanut, 117 oligonucleotide-based probes derived from genetically mapped RFLP probes were mapped onto these clones in a multiplex experimental design, and 91.5% of the overgos identified at least one BAC clone.

An alternative approach is to produce BAC libraries from diploid progenitors of peanut to eliminate problems specific to mapping a tetraploid. Recently, BAC libraries for each of the probable diploid ancestors of peanut, *A. duranensis* (A genome) and *A. ipaënsis* (B genome), have been developed (Guimarães, unpublished data). Both *Hind*III BAC libraries represent approximately 6.5 haploid genome equivalents and were constructed at CIRAD, Montpellier, France. The BAC library for *A. duranensis* contains 79,872 clones with an average insert size of 112 kb, and the *A. ipaënsis* library contains 77,184 clones with an average insert size of 100 kb.

17.5 Functional Genomics

Much of the genomics work in peanut to date has involved the development of markers, maps, and the classification of species. However, improvement of methods for gene and genome sequencing, and analysis of gene expression, promise to allow rapid strides in the identification and understanding of gene function and phenotype.

17.5.1 Gene Sequencing

Sequencing is one of the most important sources of information of genomic information. The peanut genome (2,800 Mb/1C) is large in comparison to many model plants such as *Arabidopsis* (128 Mb), rice (420 Mb), and *Medicago truncatula* (500 Mb), and is larger than soybean (1,100 Mb) and maize (2,500 Mb) (Arumuganathan and Earle 1991). The large genome size makes it unlikely that the peanut genome will be sequenced completely in the near future.

Sequencing of large numbers of expressed genes (expressed sequence tags, ESTs) can deliver substantial amounts of genetic information on protein-coding genes for comparative and functional genomics studies. As of November 6, 2006, 12,781 ESTs were deposited in GenBank. The majority were 7,454 and 2,184 sequences from seed libraries of the Chinese accessions Luhua14 and Shanyou 523, respectively, and 1,347 sequences from pod and leaf libraries of the U.S. varieties Tifrunner and C34–24. The latter are part of a larger set of 43,296 sequenced cDNA clones from 10 non-normalized peanut cDNA libraries (Luo et al. 2005a; Chen et al. 2006). From these, ca. 10,000 unique sequences have been identified. In addition, ca. 16,000 additional ESTs have been developed from normalized peanut libraries (H. T. Stalker personal communication), and smaller numbers are being sequenced by other programs.

An alternative to whole-genome sequencing is sequencing of gene-rich islands of hypomethylated DNA. A set of 1,312 genomic sequences was isolated from SSR-enriched libraries (Jayashree et al. 2005). A much larger set of sequences is under development, made by cloning of hypomethylated sequences isolated by methylation-filtration (S. J. Knapp personal communication) A set of 2,989 and 6,528 sequences from methylation-filtered and unfiltered libraries, respectively, of the cultigen, and A and B genome diploids *A. duranensis* and *A. batizocoi* have been deposited. This approach has the potential to identify genes of low expression levels that are under-represented in cDNA libraries, and to compare the A and B peanut genomes. In all, 25,259 *Arachis* sequences of all types were present in GenBank at the time of writing of this manuscript.

17.5.2 Analysis of Gene Expression

The study of gene expression has great power for associating genes with phenotype. Macroarray (nylon-based) and microarray (glass slide-based) screening methods allow for the simultaneous determination of the expression levels of thousands of genes, making it possible to attain a global view of the transcriptional state in a cell or tissue and to associate genes with functions or specific physiological conditions.

Several small scale studies have been performed. The first involved response of peanut to drought stress (Jain et al. 2001), in which 43 differentially regulated transcripts were identified by differential display. More recently, microarray technology has been demonstrated in two publications (Luo et al. 2005b, 2005c). In these,

EST-derived microarrays of ca. 400 unigenes were probed under different conditions. Twenty-five ESTs potentially associated with drought stress and response to *A. parasiticus* were identified. Likewise, 56 up-regulated transcripts were identified and confirmed by real-time PCR upon infection with *Cercospora arachidicola*. A 70-mer oligonucleotide microarray consisting of more than 10,000 gene elements is under development (Guo, unpublished results).

17.5.3 TILLING for Analysis of Gene Function and for Generation of Mutant Phenotypes

In addition to the study of gene expression by analysis of transcript levels, gene function can be studied by various types of mutational analysis or use of gene traps. The size of the peanut genome would require a large number of insertional mutant lines and an efficient transformation system, although an initial effort has been made to develop promoter traps (Anuradha et al. 2006). Targeting induced local lesions in genomes (TILLING), using ethylmethanesulfonate to generate a population of mutants and to use specific gene sequences to identify mutations, does not suffer from the same drawback because mutation frequency (number of substitutions per bp of DNA) is the key factor (Henikoff et al. 2004).

TILLING in peanut is being undertaken with the initial aim of knocking out *Ara*h2 (allergen) genes (Ozias-Akins, unpublished results). High-quality genomic sequences and an assessment of number of gene copies of *Ara*h2 were lacking until recently (Ramos et al. 2006). This information was necessary to allow the design of primers that would amplify only the A- or B-genome copy of *Arah2*, not both. After producing a pilot EMS-mutagenized population in peanut and screening 4-fold pools of 384 individuals for mutations in *Arah2*, the mutation frequency appears to be comparable to that achieved in *Arabidopsis*, i.e., 1/170 kb (Greene et al. 2003). Variations in mutagen treatment currently are being tested to scale up the size of the population. For *Arabidopsis*, a population of ca. 6,900 mutants is available, but a sufficient number of mutations (ca. eight per gene) was found after screening about half that number (Greene et al. 2003).

17.5.4 Transformation

Transformation and regeneration are important components of the success of future genomics work in peanut. A high-throughput system is needed for determination (or confirmation) of function of the rapidly-expanding number of gene sequences. In addition, transformation is instrumental in development of transgenic plants with important value-added traits.

Regeneration of genetically engineered peanuts was accomplished in 1993 (Ozias-Akins et al. 1993). *Agrobacterium tumefaciens*-mediated transformation,

typically performed on shoot-forming cultures using neomycin phosphotransferase (*nptII*) as a selectable marker, is characterized by strong genotype specificity for Valencia-type peanuts (Cheng et al. 1996). The advantage of *Agrobacterium* transformation is the higher frequency of low-copy number inserts (Sharma and Anjaiah 2000) and a reduced amount of gene silencing. Microprojectile bombardment of somatic embryos using hygromycin phosphotransferase (*hph*) as a selectable marker is the most broadly applicable method for peanut by virtue of working across genotypes and has been performed by multiple groups. Although high copy number and gene rearrangements remain more common with bombardment, regenerated lines with low-copy number inserts can be selected by Southern blot analysis.

Concerns regarding peanut transformation/regeneration for genomics and varietal development include low efficiency, sterility of some regenerants, and the 12 to 18 months required for the process (Egnin et al. 1998). The use of antibiotic resistance genes is a concern for consumer acceptance, but it is possible to visually select for transformed embryogenic tissues expressing green fluorescent protein (Joshi et al. 2005).

Numerous genes have been introduced into peanut (Ozias-Akins and Gill 2001; Ozias-Akins 2005), and most involve disease resistance. Coat protein-mediated virus resistance was shown first in tobacco (Powell et al. 1986), and subsequent research has shown pathogen-derived resistance to be applicable to many viruses and crop species. Greenhouse and field studies have shown that peanut lines expressing a sense or antisense nucleocapsid protein gene of TSWV can show elevated levels of tolerance to virus infection (Li et al. 1997; Magbanua et al. 2000; Yang et al. 2004). A similar strategy resulted in resistance to peanut stripe potyvirus (Higgins et al. 2004) and peanut clump virus (see Dar et al. 2006).

Peanut production also suffers from several fungal diseases. Chitinase, glucanase, or oxalate oxidase genes have been introduced to target sclerotinia blight (Chenault et al. 2005; Livingstone et al. 2005). The maize *RIP* 1 gene has been reported to reduce aflatoxin in transgenic peanut (Weissinger et al. 2006). Some strategies for reducing problems caused by fungi are indirect. For example, the insect pest the lesser cornstalk borer can cause damage to peanut pods and in the process also inoculate pods with *Aspergillus*. A reduction in pod damage due to the expression of *cryIA*(c) may also reduce pod infection with *Aspergillus* and consequent aflatoxin production (Ozias-Akins et al. 2002). In addition, attempts are underway to introduce the dehydrin-responsive element (DREB) element for drought resistance, which could also reduce aflatoxin contamination (see Dar et al. 2006).

17.6 Proteomics and Allergen Genes

Food allergies are common in the population, and involve various foods, including fish, milk, eggs, soybean, and tree nuts. Some of the major peanut allergens are seed storage proteins. Conarachin is known as *Ara*h1, a vicilin, and genes for expressing this protein have been described (Burks et al. 1995). Similarly, genes for arachin

(glycinin, *Ara*h3) (Rabjohn et al. 1999) and *Ara*h2 (conglutin) (Stanley et al. 1997) have been cloned and sequenced. *Ara*h1 and *Ara*h2 are considered to be the major allergens because they are recognized by serum IgE from > 90% of peanut allergic individuals (Burks et al. 1998). *Ara*h1 and *Ara*h3 probably are encoded by multigene families, whereas *Ara*h2 has been shown to derive from two genes in cultivated peanut, one from each genome (Ramos et al. 2006). *Ara*h4 through *Ara*h7 are other seed proteins, present in smaller amounts (Kelber-Jancke et al. 1999). Up to six additional allergens have been identified (De Jong et al. 1998).

High-resolution 2-D gels combined with matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) analysis is being applied to the characterization of allergens, including peanut allergens (Law et al. 2005; Boldt et al. 2005). Several accessions have been found to lack one or more allergen subunits (Liang et al. 2006). These methods are essential for the manipulation of peanut seed proteins by mutagenesis or transgenic approaches.

17.7 Biodiversity and Markers

One of the strengths of peanut is the large number of accessions in germplasm collections (Holbrook and Stalker 2002). These harbor sources of alleles that have been underused. Development of core collections is a major emphasis, and application of genomics to these is expected to enhance the use of these resources.

17.7.1 Collections

There are four major peanut germplasm collections in the world. The largest collection consists of 14,966 accessions of cultivated and 453 accessions of 44 wild *Arachis* species from 93 countries; this collection is housed at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Other major holders are the USDA Southern Regional Plant Introduction Station, Griffin, GA, USA (9,027 accessions), the National Research Center for Groundnut, Junagadh, India (7,935 accessions), and the Chinese national collection (5,890 accessions).

17.7.2 Assessing Phenotypic Diversity and Enhancing Germplasm Use

Core collections (ca. 10% of the entire collection) of large germplasm collections have been established for greater ease of screening. These include 1,704 (ICRISAT), 831 (US), and 582 accessions (China) (Upadhyaya et al. 2003; Holbrook et al. 1993; Jiang et al. 2004). The phenotypic diversity of the ICRISAT core and important descriptor traits were determined by Upadhyaya et al. (2003) Evaluation was

performed for 16 morphological and 15 agronomic characters during the rainy and post-rainy seasons. The average phenotypic diversity index was higher in the *fastigiata* group (0.146) than the *hypogaea* group (0.141), but the maximum phenotypic diversity (0.453) was observed between two *hypogaea* accessions. The two subspecies differed significantly for all traits except leaflet surface and oil content. Principal coordinate and principal component analysis showed that 12 morphological descriptors and 15 agronomic traits were important in explaining multivariate polymorphism.

Evaluation of the U.S. core resulted in identification of resistance to root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood race 1 (Holbrook et al. 2000), tomato spotted wilt (Anderson et al. 1996), cylindrocladium black rot [*Cylindrocladeum crotalariae* (Loos) Bell and Sobers] and early leafspot (*Cercospora arachidicola* Hori) (Isleib et al. 1995), rhizoctomia limb rot (*Rhizoctonia solani* Kuhn) (Franke et al. 1999), sclerotinia blight [*Sclerotinia minor* Jagger], and reduced preharvest aflatoxin contamination (Holbrook et al. 1998).

To further facilitate the use of germplasm accessions, Upadhyaya, et al. (2002) and Holbrook and Dong (2005) developed peanut minicore collections (1% of entire collection) consisting of only 184 and 111 accessions, respectively. The evaluation of the ICRISAT core and minicore collections led to identification of new sources for drought tolerance (Upadhyaya 2005) and early maturity (Upadhyaya et al. 2006a). Preliminary evaluation of the U. S. minicore has identified new sources of heat tolerance (Kottapalli, unpublished results).

Wild species are also an important potential source of alleles. Alleles for strong resistance to various diseases and pests are present in wild species, including root-knot nematodes (*Meloidogyne arenaria* (Neal) Chitwood), early leafspot (*Cercospora arachidicola* Hori), late leafspot (*Cercosporidium personatum* (Berk. et Curt.) Deighton), rust (*Puccinia arachidis* Speg.), groundnut rosette virus, tomato spotted wilt virus, peanut stunt virus, peanut mottle virus, and lesser cornstalk borer (*Elasmopalpus lignosellus* Zeller) (Stalker and Moss 1987).

17.7.3 Use of Markers for Measuring and Using Diversity

Efficient use of germplasm collections would be improved by identification of accessions possessing different alleles for specific traits and by development of selectable markers for breeding.

Recent studies have disproved the belief that cultivated peanut lacks variation at the molecular level (He and Prakash, 1997; Subramanian et al. 2000). SSR markers were shown to be useful for detecting diversity in cultivated peanut and can be used for population studies (Moretzsohn et al. 2004; Ferguson et al. 2004a). ICRISAT is using SSR markers to analyze genetic diversity in cultivated germplasm resistant to late leafspot, rust, and bacterial wilt (Mace et al. 2006, 2007). In some cases, more than half of the markers detected polymorphism with polymorphism information content (PIC) values of over 0.5. A more elaborate study

involving about 1,000 accessions and 21 SSRs revealed 491 alleles (5–46 alleles/locus) (Upadhyaya et al. 2006b). The mean PIC value was 0.796, and *fastigiata* and *hypogaea* subspecies formed different clusters. The 184 minicore accessions accounted for about 75% alleles of the cultivated accessions in the composite collection. However, wild species had greater diversity: 52 accessions of 14 wild *Arachis* species had more alleles (373) than 333 accessions of *hypogaea* (308 alleles) and 365 *fastigiata* accessions (365 alleles).

Likewise, analysis of the U. S. core subset demonstrated considerable molecular diversity (Kottapalli et al. 2007). Moderate levels of genetic variation were found with genetic distances (D) values among accessions ranging from 0.088 to 0.254. Seventy-two primers amplified 528 bands. PIC values ranged from 0.027 to 0.375, with an average value of 0.15, and from two to 28 polymorphic bands per primer were observed. Distinct groupings of the accessions were observed based on subspecies, and runner/Virginia, Spanish, and Valencia market types were clearly distinguished for approximately 90% of the accessions tested. Twelve of the markers, mapped previously to the A genome, were found sufficient to identify subspecies and botanical types and gave a clustering pattern very similar to the entire 67 SSR marker set.

17.8 Bioinformatics

One of the important needs for genomics has been the informatics resources for analysis of the large amounts of data produced and for comparison of data among species. A map database for peanut, called PeanutMap, has been published recently (Jesubatham and Burow 2006). Peanut Map contains the published maps of the peanut genome, plus smaller map sets of markers associated with traits. The database software allows comparison among linkage groups in a map, showing marker correspondences among homoeologous chromosomes, as well as among maps from different publications.

As awareness of the significance of comparative genomics has increased; databases encompassing data from multiple species are being created. A cross-legume database, called the Legume Information System, has been released to incorporate species-specific data and permit cross-legume comparisons (Gonzales et al. 2005). This will permit data held in different legume databases to be used for comparison of synteny among different species. Legume Information System also incorporates sequence data, making it possible to search for genes expressed in different tissues and at different physiological conditions.

17.9 Perspective

Peanut genomics is beginning to make the progress needed for greater utility in genetic improvement of the species. Advances in peanut have been hindered by a large genome, apparent lack of polymorphism in the cultigen, difficulty in interspecific

gene transfer, and a slow transformation/regeneration system the successes of which have been limited by the lack of marketability of transgenic peanut.

These and other limitations are changing for several reasons. There is increased realization of the genetic variability present in the genus, both in the cultigen and wild species. Development of SSR markers has been successful in identifying this variation, making marker work in both the cultigen and wild species feasible. The development of tools in parallel for the cultigen and A and B genome diploids will help in simplifying the work of understanding the organization and evolution of peanut. Mapping of simple polymerase chain reaction-based markers will assist with paving a way for QTL fine mapping and efficient MAS. There is still an unmet need for SNP-based maps and markers. Combined with recent theoretical developments in linkage disequilibrium and genetic association mapping, additional options may be available for identification of biotic and abiotic stress tolerance markers.

Construction of multiple cDNA and genomic libraries and the beginnings of significant sequencing are making data available that can be used for multiple projects. Identification of EST unigene sets and genomic sequences will be useful, especially in comparison with sequencing of the *Medicago* and soybean genomes. Sequence matching of peanut genes with other genomes will identify putative orthologous loci and facilitate transfer of information on gene function, and because of colinearity among legume genomes, mapping of genes can be extended to other species.

Work on TILLING, transformation, and gene expression analysis will assist with the understanding of gene function. TILLING has the potential to overcome some of the disadvantages of the large peanut genome size, and development of transformation-competent BAC libraries and more-efficient transformation systems would assist with studies of gene function. Identification of critical gene pathways and specific genes will be useful for conventional and transgenic improvement programs.

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