Chapter 3

RECENT METHODOLOGIES FOR GERMPLASM ENHANCEMENT AND BREEDING

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INTRODUCTION

Peanut cultivars currently available to growers in the U.S. are the result of extensive selection and breeding activities to combine many desirable characteristics into single genotypes. Breeding efforts have been in place for over 60 years at public institutions in Florida and North Carolina (Knauft *et al.*, 1987); and long-term programs exist with state and/or federal support in Georgia, Oklahoma, and Texas, and at the private firm, AgraTech Seeds, Inc. Peanut breeding efforts have been reduced in recent years, with elimination of the state-supported breeding program at New Mexico State University, loss of USDA peanut breeding positions (Stillwater, OK; Suffolk, VA; and Auburn, AL), and elimination of the peanut breeding program based on the campus of the University of Florida.

While earlier efforts at cultivar development frequently relied on selection of genetic variability within landraces, most of the recent releases are the result of several cycles of crossing and selection (Knauft and Gorbet, 1989b; Isleib and Wynne, 1992). These efforts have resulted in peanut cultivars with high yield potential, suitable maturity, some pest resistance, uniform pod and seed size, and desirable processing characteristics for the shelling and manufacturing industry. Current cultivars have provided economic benefits to growers, as well as to shellers and manufacturers, and have given the public high quality peanut products at an affordable price.

The challenge facing breeders of future cultivars is to maintain the desirable levels of existing characteristics while improving other aspects of the plant. A number of traits are focal points of genetic research and breeding activities for cultivar improvement. Public concern over pesticide residues in food and pesticide contamination of the environment, as well as increased costs and reduced efficacy of farm chemicals, provide incentive for increasing the already considerable efforts to develop pest resistance in peanut. Changes in seed composition also may be beneficial. If meaningful decreases in oil content can be made, lower-calorie products may be developed for the snack food industry. Corresponding increases in oil content may improve the already substantial oil yield per ha and increase its use as a vegetable oil or perhaps even as a diesel fuel substitute after ethyl esterification. Improvements in the quality of oil, protein, or carbohydrate may improve market share of peanut products as well.

U.S. peanut production has received a governmental price support since the late 1930s. This support has allowed the development of a production system for peanut growers that results in a high quality peanut crop and an opportunity for profit. Peanut cultivars have been developed in the context of production with inputs based on economic return as a function of this price support. Given the uncertain future of continued governmental backing of this support system, U.S.-grown peanuts must become more competitive on the world market. Thus, breeders also must consider the need to develop cultivars that can produce an economic return when grown with reduced economic inputs. Besides increased pest resistance, physiological traits such as early season vigor, competitiveness with weeds, drought tolerance, early maturity, more determinant growth habit, and more efficient plant characteristics for rapid drying of pods may become important.

Most of these characteristics are desirable in peanut cultivars grown anywhere in the world. However, because purchased inputs for peanut production are often economically less viable in developing countries, cultivars may need greater levels of stress resistance. Very early maturity, which has a yield cost in peanut, may be viable in some systems where peanuts are part of multiple cropping systems or where such maturity may provide escape from biotic or abiotic stresses. In many peanut-producing countries, peanuts are intercropped and the development of specific types for these conditions may be appropriate as well.

Peanut breeders have at their disposal three major sources of genetic variability for obtaining the needed gene sequences for expression of desired characteristics. Variability available or inducible within *Arachis hypogaea* L. or *A. monticola* Krapov. et Rigoni can be incorporated into existing cultivars and breeding lines through artificial hybridization. Diversity within other *Arachis* species is less accessible, but introgression may occur through utilization of a range of procedures described by Stalker and Simpson (see Chapter 2). Molecular techniques now allow theoretical access to unlimited genetic variability from any living organism or artificial construct.

TECHNIQUES TO USE THE EXISTING VARIABILITY OF *A. HYPOGAEA* FOR PEANUT CULTIVAR IMPROVEMENT

Genetic Resources and Their Evaluation

Existing genetic resources of *A. hypogaea* are extensive. The U.S. Department of Agriculture (USDA) has a long-term repository at the National Seed Storage Laboratory in Ft. Collins, CO. The USDA collection at the Southern Regional Plant Introduction Station in Griffin, GA is an active repository that allows routine access of its germplasm by peanut scientists. The Center has over 7000 accessions of *A. hypogaea* (Holbrook *et al.*, 1993) and material continues to be supplemented by additional resources collected on plant exploration trips sponsored by the USDA and the International Board for Plant Genetic Resources (IBPGR) (Simpson, 1991). In most cases,

germplasm from recent trips has been deposited in both the USDA germplasm system and in the Genetic Resources Unit of the International Crops Research Institute of the Semi-Arid Tropics (ICRISAT), Hyderabad, India. Trips made during the last 15 years have been undertaken with an awareness that genetic resources from a country represent a valuable commodity for all parties involved in exploration. Over 1900 cultivated accessions have been collected during this time and, in almost all cases, duplicate samples are deposited in collections of the source country (Simpson, 1991). This allows enhancement of germplasm resources in the host country, as well as in the U.S. plant introduction system and the Genetic Resources Unit of ICRISAT.

Additional genetic resources exist in the breeding programs of Texas A&M University (College Station and Stephenville), Oklahoma State University, North Carolina State University, the University of Georgia (both state and USDA), the University of Florida (Marianna), and AgraTech Seeds, Inc. (Ashburn, GA). While some materials at these research sites are duplicates of plant introductions available from the Southern Regional Introduction Station, many unique breeding lines generated from artificial hybridization and selection are stored at these facilities. The lines represent non-commercializable breeding lines, genetic stocks, materials from genetic studies, and germplasm developed to incorporate tolerance to various biotic and abiotic stresses. As a whole, they represent an important additional germplasm resource.

The most extensive peanut germplasm collection, that of ICRISAT in India, contains about 13,000 accessions. The extent of duplication between the USDA and ICRISAT collections is not known, but has been estimated to be between one-third and one-half the ICRISAT collection (R.N. Pittman, pers. commun., 1993). The collection at ICRISAT has been developed to provide not only long-term storage for peanut germplasm, but also to provide materials for breeding programs, particularly in the developing world. Germplasm collections exist in many other countries, including Argentina, Australia, China, South Africa, Israel, Taiwan, Nigeria, Malawi, Zimbabwe, and Senegal. While germplasm flow between countries can be straightforward, phytosanitary regulations and political considerations often slow the rate at which germplasm circulates.

The germplasm collections have been utilized by breeders and geneticists in two primary ways. The material may be screened to characterize unknown variability. The ICRISAT collection has been examined for pest and disease resistance, as well as drought tolerance (Reddy, 1988), and the USDA collection has been screened for resistance to late leaf spot [*Cercosporidium personatum* (Berk. & Curt.) Deighton] and peanut root knot nematode [*Meloidogyne arenaria* (Neal) Chitwood] (C.C. Holbrook, pers. commun., 1993). Costs of screening the entire USDA or ICRISAT collection can be prohibitive for many characteristics. Holbrook *et al.* (1993) identified a subset of the USDA collection using a random sample of groupings stratified by country of origin and available morphological data. The information was analyzed using multivariate statistical analysis. Results allowed accessions to be clustered into groups which, theoretically, are genetically similar. Random

sampling was then used to select approximately 10% of the lines from each group. Examination of data for six phenotypic traits indicated that the genetic variation expressed for each trait in the entire collection has been preserved in this core collection (Holbrook *et al.*, 1993). The core collection may be used as a working group of plant introductions for extensive evaluation of characteristics, as well as for identification of clusters containing a particular trait of interest. A study comparing use of the entire collection as opposed to using the core collection to identify late leaf spot resistant lines found that the core collection could improve efficiency of germplasm evaluation (Holbrook and Anderson, 1992). Although it contained only 10% of the entries in the entire collection, 54% of the known late leaf spot-resistant accessions were identified. The core collection also has been used to screen for variation in oil content (J. Bruniard, C. C. Holbrook, and D. A. Knauft, 1993, unpubl. data). The range of this trait was found to be similar to that found in the cultivated species (Salunkhe *et al.*, 1992).

As accessions within collections are characterized, the data gathered are compiled into catalogs. This information can be accessed by scientists, and plant introductions containing desired characteristics may be obtained for research purposes or cultivar development. Development of the Germplasm Resources Information Network (GRIN), a data base of descriptor information for each plant introduction in the USDA system, has made it more efficient to access information regarding this collection. A pcGRIN version is available for use on a microcomputer (USDA, 1992).

Genetic Variability

Despite the considerable genetic resources of peanut, the genetic information available for peanut is limited when compared to that of many other crops of economic importance. Although the inheritance of many traits has been described (Reddy, 1988; Wynne and Halward, 1989), further genetic information is limited. For example, few linkage groups have been reported. Four were cited by Halward *et al.* (1991a), Murthy *et al.* (1988) found linkage among four genes for pod shape, and linkage of 7.8 recombination units between loci for purple testa and orange corolla color was identified by Knauft *et al.* (1991a). Few molecular markers have identified polymorphisms in *A. hypogaea*. Little isozyme variability has been reported (Grieshammer and Wynne, 1990a; Lacks and Stalker, 1993) and few restriction fragment length polymorphisms (RFLP) have been found in cultivated peanut, whether using genomic probes (Kochert *et al.*, 1991; Ro *et al.*, 1992) or single primers of arbitrary sequence (Halward *et al.*, 1991a, 1992). There have been limited reports of peanut gene isolation and sequencing (Buffard *et al.*, 1990; Abbott *et al.*, 1991; Zeile *et al.*, 1993b).

Establishing an aneuploid chromosome series will be necessary before genes can be associated with specific chromosomes. Aneuploids have been derived from interspecific hybrids, but these are generally unstable and the origin of extra chromosomes, either derived from wild species or the domesticated peanut, is unclear (see Stalker, 1985a, Singh *et al.*, 1991). In *A. hypogaea*, several aneuploids have been reported (see Stalker, 1985b;

Smartt, 1990 for reviews). Small seeds of virginia-type peanuts are aneuploid at a relatively high frequency, and eight different trisomics or double trisomics have been identified (Stalker, 1985b). Banks and Kirby (1990) reported a sterile monosomic plant and Singh and Joshi (1981) reported a nullisomic plant with 19 bivalents, which was also highly sterile. Attempts to produce an aneuploid series for *A. hypogaea* will take a considerable amount of effort; and the reports of highly sterile monosomic and nullisomic plants may mean that haploidy via anther culture or other techniques may not be useful for peanut.

Genetic variability and the inheritance of many economically important traits are discussed in other chapters of this book. Host plant resistance to insects is covered by Lynch and Mack in Chapter 4, Chapter 5 encompasses host plant resistance to diseases by Sherwood *et al.*, plant genetic differences in response to weeds are mentioned by Wilcut *et* al. in Chapter 6, and manipulation of the physiology of the peanut plant is discussed by Williams and Boote in Chapter 9.

Testa Color. The inheritance of testa color perhaps has been studied more extensively than any other trait in peanut (Reddy, 1988; Wynne and Halward, 1989). The relative ease of evaluation, the wide range of discrete variability, and the importance of specific testa colors and color uniformity within cultivars contribute to the thorough genetic description of this trait. Many studies confirm that, although peanut is an allotetraploid, the two genomes contain a significant amount of duplicate information. For example, after a dominant gene for white testa color was identified (Norden *et al.*, 1988), a dominant gene was identified in an additional source (Branch, 1989). Tests for allelism between the two sources found them to be unique genes (Knauft *et al.*, 1991a). A second gene for red testa color also has been identified after the initial gene was studied (Holbrook and Branch, 1989).

Leaflet Shape. Many leaflet shapes have been described in peanut (Reddy, 1988). Commercial utility has not been reported for these mutants. Nevertheless, information on their genetic control can allow them to be used as markers. For example, the dominant gene, krinkle, has been used frequently for outcrossing studies (Coffelt, 1989a; Knauft *et al.*, 1992). Other leaflet mutants include curly-leaf, controlled by a single recessive gene (Branch, 1987), and puckered leaf, which segregated in an F_2 ratio of 13 normal to 3 puckered (Dwivedi and Nigam, 1989).

Pod Yield. Although Wynne and Coffelt (1982) concluded that additive gene effects were most important for most traits in peanut, a number of subsequently published studies (Sangha and Labana, 1982; Green *et al.*, 1983; Swe and Branch, 1986; Dwivedi *et al.*, 1989; Bansal *et al.*, 1991) found nonadditive gene effects for pod yield to be equal to or greater than additive effects. On the other hand, Halward and Wynne (1991) found additive effects to be significant for pod and seed weight in two diverse crosses, while nonadditive effects were inconsistent.

Seed Composition. Peanut seed are composed of several major components, including oil, protein, carbohydrates, minerals, and vitamins.

a. Oil. The oil content of peanut seed has been reported to range from

35.8-54.2%, and averages near 45% (Bovi, 1983; Jambunathan *et al.*, 1985, 1993; Raheja *et al.*, 1987; Dwivedi *et al.*, 1990; Salunkhe *et al.*, 1992). Although peanut is grown primarily for vegetable oil production throughout the world, the oil makes an excellent substitute for diesel fuel. The crop produces over three times the energy required for growth, and in Virginia this energy is produced on 25 to 30% of the area required to produce the same amount of energy in a soybean [*Glycine max* (L.) Merrill] crop (Coffelt, 1989b).

Ironically, there may be economic value in reducing the oil content of peanut for confectionery use. Lines with an oil content of 35% or less may have potential use in the development of lower calorie snack foods. Breeding lines exist in the Florida program that produce seed with near 20% oil (Jakkula *et al.*, 1993). The trait shows variable expressivity on individual plants within true-breeding lines, as seed having oil contents ranging from 20% to 48% are found on the same plant. At maturity the low-oil seed have a higher water content than normal peanut seed, resulting in shriveling after postharvest drying. Seed yields of these lines are not as high as standard cultivars, and the shriveled seed may limit consumer acceptability.

Because peanut has such a high oil content, the composition of the oil plays a major role in defining peanut quality. Peanut oil contains eight fatty acids in proportions of 1% or more. Significant variation has been found in *Arachis* spp. for fatty acid composition, particularly for larger quantities of long-chain fatty acids (Stalker *et al.*, 1989). In a study of over 450 genotypes of cultivated peanut, significant genetic variation was noted for all eight fatty acids (Norden *et al.*, 1987). Other studies with more limited germplasm (Treadwell *et al.*, 1983; Raheja *et al.*, 1987; Branch *et al.*, 1990) reported fatty acid contents within the ranges reported by Norden *et al.* (1987). Because palmitic (16:0), oleic (18:1), and linoleic (18:2) acids together constitute 90% of most peanut oil, variation among these fatty acids is most important for conferring different physical properties and shelf-life qualities. Breeding lines with less than 6.5% palmitic acid, more than 80% oleic acid, and near 2% linoleic acid have been identified (Norden *et al.*, 1987; Knauft *et al.*, 1993b). These lines show greater oxidative stability than normal peanut lines (O'Keefe *et al.*, 1993), while the compositional change has little effect on texture, aroma, or flavor (Moore *et al.*, 1989). The high oleic acid trait also may have desirable physiological effects on animals and humans. When fed to swine, the line caused increased levels of unsaturated fat in pork without affecting sensory characteristics of the meat (Myer *et al.*, 1992). The same peanuts were part of a low-fat diet that improved serum lipid profiles of women (O'Byrne *et al.*, 1993). The high oleic/low linoleic acid characteristic is controlled by two recessive genes (Moore and Knauft, 1989), and one of the two genes appears to be common in peanut germplasm (Knauft *et al.*, 1993b). Except for the simple inheritance of this fatty acid compositional change, other reported variability for peanut fatty acids appears to be quantitative, with general combining ability consistently more important than specific combining ability (Sykes and Michaels, 1986; Mercer *et al.*, 1990).

b. Protein. Peanut protein content has been reported to range from 16.2

to 36%, with most genotypes averaging near 25% (Dwivedi *et al.*, 1990; Salunkhe *et al.*, 1992). Protein content has a significant inverse correlation with oil (Dwivedi *et al.*, 1990). Like oil composition, genetic variation also exists for the structure of peanut protein. However, fewer studies have been conducted on protein composition, likely because peanut is not a major dietary source of protein for humans or animals. It has been suggested that the nearly 4.5 million t of peanut protein harvested each year represent a significantly under-utilized nutrition source (Singh and Singh, 1991).

Krishna *et al.* (1986) identified electrophoretic variation in one of the major peanut storage protein fractions, arachin, although no variation was detected in the other major fraction, conarachin. Genetic differences also exist for amino acid composition in peanut protein, although most variability has been found in *Arachis* species other than *A. hypogaea* (Basha and Pancholy, 1984; Bianchi-Hall *et al.*, 1993). Differences in protein nutritional quality and composition have been found in some studies (Ghuman *et al.*, 1990; Kim *et al.*, 1992), while others have shown little significant variation in protein quality among peanut genotypes (Basha, 1992b; Jambunathan *et al.*, 1992). The conflicting reports from these studies are likely the result of the varying genetic base used by the researchers. Although significant, the differences are not of a magnitude to allow meaningful change in the methionine content, which is a limiting dietary amino acid in peanut and other legume species.

An important aspect of peanut seed composition that has received little breeding or genetic attention is its allergenicity. Peanuts are the major allergenic food among adults in the U.S. and are one of the leading allergenic foods among children (Taylor, 1992). Reactions range in symptoms from skin rash to fatal anaphylactic shock. Most of the allergens are unidentified, although allergen activity is present in the two major storage proteins in peanut, arachin and conarachin. It is not known to what extent allergic responses could be reduced through either conventional or molecular genetic manipulation of peanut proteins.

c. Carbohydrates. The amount of carbohydrate ranges from 10-20% of whole peanut seed and genetic differences have been reported (Salunkhe *et al.*, 1992). Starch (12.5% of defatted meal) and sucrose (14% of defatted meal) are the major carbohydrate components found. Genetic variation has been identified for starch and sucrose content (Gupta *et al.*, 1982; Basha, 1992a). In spite of known differences, genetic manipulation of carbohydrates has received little attention from peanut breeders, most likely the result of the relative difficulty of analysis and the lack of clear economic advantages to manipulations.

d. Minerals and vitamins. Genetic variation in mineral composition of peanut seed has been identified (Branch and Gaines, 1983; Jambunathan *et al.*, 1992). Although peanuts can be a significant source of calcium, phosphorus, and iron, as well as the vitamins thiamine, riboflavin, and niacin (Coffelt, 1989b), little effort has been made to improve this aspect of peanut seed composition.

Flavor. The roasting of peanut seed produces a complex reaction between

amino acids, a peptide, and carbohydrates (Newell *et al.*, 1967). The resulting nutty aroma and flavor are largely responsible for the widespread use of peanut seed in cooking, production of peanut butter, and its use singly or as a component in snack food. Improvement in peanut flavor may be possible as genetic differences in flavor have been identified (Pattee and Giesbrecht, 1990; Jambunathan *et al.*, 1993).

Seed Size Distribution. Uniformity of seed size is a desirable attribute in a peanut cultivar. Uniform seed size improves efficiency of planting, shelling, blanching, and roasting, increases the percentage of usable seed for specific peanut products, and is important for consumer acceptance of some products. Seed size is associated with both quality and value of the crop. Genetic variation for seed size uniformity has been reported (Knauft *et al.*, 1991b). While uniformity generally decreased with increasing seed size, within market-type variation was not related to size. Size uniformity was consistent in different environments, suggesting that genetic improvement in this trait could occur if economically beneficial.

Agronomic Seed Quality. The previous discussion of peanut seed composition was based on the desirability of genetic changes for improved aspects of edible quality. Compositional differences also may affect agronomic seed quality. In a study of seed storage, Ketring (1992) found genetic differences in germination, as well as in field emergence both within and across seed storage periods. Given the need for improved seed quality in peanut, this area of research deserves additional attention.

Breeding

Breeding procedures for peanut differ little from those used for other self-pollinated crops. Several chapters have been published on breeding techniques (Knauft *et al.*, 1987; Reddy, 1988; Coffelt, 1989b; Nigam *et al.*, 1991), and the following discussion will be limited to reports of recent research activity. Although procedures vary, all follow similar steps to cultivar development. Appropriate parents are chosen to meet the desirable objectives of the breeding program and are artificially hybridized. Segregating generations are advanced until progeny from individual plants breed true to type, selection is practiced to obtain breeding lines expressing the desired objectives, and the lines are evaluated in comparison to standards.

Parent Selection. In the last 30 years a large portion of U.S. peanut production has come from relatively few cultivars. Several cultivars, including Florunner (U.S. runner market type, *A. hypogaea* subsp. *hypogaea* var. *hypogaea*), Florigiant (U.S. virginia market type, *A. hypogaea* subsp. *hypogaea* var. *hypogaea*), and Starr/Comet (U.S. spanish market type, *A. hypogaea* subsp. *fastigiata* var. *vulgaris*, with Comet a selection from Starr), predominated peanut production from the time of their release through the early 1980s. These cultivars, when combined with a package of inputs made available to growers at that time, gave substantial yield increases when compared to previous cultivar/input packages. By 1974, Florunner was grown on 99.5% of the area devoted to runner market-type production, Starr and Comet were grown on 82.5% of the spanish area (Hammons, 1976), and

Florigiant was grown on over 80% of the virginia area (T.A. Coffelt, pers. commun., 1993). Each cultivar, and especially Florunner, became the industrial standard for the market type with regard to pod and kernel size, shelling percentage, blanching and roasting attributes, protein, carbohydrate, and oil content and composition, and flavor. The manufacturers of peanut food products developed manufacturing procedures based on these standards. When newer cultivars were grown, they were co-mingled with the existing predominant cultivar at sheller sites and were provided as a mixed product to the food industry. Because of this co-mingling, breeding lines were released as new cultivars only if they maintained similar levels of expression of these important manufacturing traits to prevent processing inconsistencies.

This need to maintain existing characteristics has influenced the choice of parents in peanut breeding programs. Unless a predominant cultivar, or phenotypically similar line, is used as a parent in crossing, the chance is small that offspring will be obtained that combine the desired expression of the many economically important characteristics. As a result, although a number of peanut cultivars have been released since the mid-1970s, most have been related to Florunner, Florigiant, and Starr/Comet (Knauft and Gorbet, 1989b; Isleib and Wynne, 1992). As late as 1989, four ancestral lines, Small White Spanish, Dixie Giant, Spanish 18-38, and Basse, contributed most of the genetic composition of runner peanut cultivars (Isleib and Wynne, 1992). Recently released runner cultivars, such as Marc I, Andru 93, AT 127, Georgia Runner, and Georgia Browne, are genetically more diverse than previous releases and should widen the genetic base of this market type if they are accepted by growers. Contributions of ancestral lines to the genetic composition of virginia and spanish cultivars are more varied than for runner cultivars (Isleib and Wynne, 1992). Nevertheless, researchers still consider the genetic base of peanut cultivars to be narrow.

Market constraints and genetic similarity provide the peanut breeder with a dilemma when choosing parents for crossing. The breeder wishes to increase diversity both among parents and among cultivars emanating from the program. Yet increasing such diversity will likely reduce the chances of obtaining the desired set of characteristics in a given line. Only the backcross method and transformation techniques allow maintenance of the desired array of traits in an existing cultivar or advanced breeding line while moving a small amount of genetic material from a donor line. Given the limited number of simply inherited, economically important traits in peanut, the current climate surrounding regulatory issues, and some public resistance to the use of transgenic plants, the two methods have obvious limitations.

Other breeding procedures rely on selection of lines from segregating generations that contain an initial combination of 50% genetic material from each parent in the F_1 generation. Therefore, the utilization of unadapted material for incorporation of traits such as pest resistance or quality factors introduces large quantities of undesirable genetic material.

Given these conditions, choosing appropriate parents for cultivar development can improve the efficiency of a program. Several methods have been evaluated for identification of desirable parental lines. Researchers

using both testcrosses (Isleib and Wynne, 1983b) and diallel analysis (Holbrook, 1990) concluded that parental performance *per se* could be used to identify desirable parents for grade parameters and yield. However, other studies using line x tester analysis found that high mean performance for pod yield and other characteristics among lines or testers frequently was associated with low general combining ability (Upadhyaya *et al.*, 1992).

Increasing diversity is an appropriate goal in breeding programs given the narrow genetic base of current cultivars (Knauft and Gorbet, 1989b). Durga Prasad *et al.* (1985) used cluster analysis to choose diverse peanut lines for parents in a breeding program. With this procedure they were successful in identifying distinct lines for crossing that maintained a common desirable bunch plant type.

F_1 performance also may be useful for predicting parent potential, particularly if heterosis is caused by epistatic gene effects that can be captured efficiently in segregating generations. Isleib and Wynne (1983a) showed the largest amount of heterosis occurred in crosses between parents with the greatest amount of genetic diversity. Arunachalam *et al.* (1984) found greatest heterosis for yield in crosses with intermediate diversity. Several studies showed that crosses with greater diversity had dominant gene action for pod yield, while crosses between more closely related lines exhibited gene action that could be effectively selected in earlier generations (Isleib and Wynne, 1983a; Halward and Wynne, 1991). While giving the greatest heterosis, largely divergent parents may not be the most appropriate basis for choosing parents to improve diversity or to incorporate desired traits into peanut. F_1 performance *per se* was found to be ineffective for selecting desirable parental combinations (Isleib and Wynne, 1983b; Holbrook, 1990).

Reciprocal Cross Differences. In addition to the choice of parents, the direction of the cross also can be a factor in determining the type of segregants obtained. Wynne and Halward (1989) summarized cytoplasmic effects reported to that time and indicated that in most instances it was observed in crosses between spanish and virginia botanical types. More recent reports have substantiated that many reciprocal differences are the result of intersubspecific crosses. Branch and Kvien (1992a) identified cytoplasmically inherited albino seedlings from three intersubspecific crosses between spanish and virginia botanical types. These authors also noted nuclear inheritance of albinism that showed no reciprocal differences. Other studies have found similar noncytoplasmic inheritance for albinism (Dwivedi *et al.*, 1984). Essomba *et al.* (1991) studied stem color in peanut and found a complex inheritance pattern. They suggested extranuclear factors may be either allelic to nuclear genes or may modify their expression. Reddy *et al.* (1988) found many reciprocal cross differences in a 6 x 6 diallel among spanish, valencia (*A. hypogaea* subsp. *fastigiata* var. *fastigiata*), and virginia botanical types. They indicated higher yields may be obtained generally from using spanish lines as female parents in crosses with other botanical types. This study did not distinguish true cytoplasmic inheritance from maternal effects, however, since analysis was conducted only on F_1 plants.

Instances of maternal effects have been reported in inheritance studies on seed dormancy (Khalfaoui, 1991) and fatty acid composition (Mercer *et al.*, 1990). Such effects are not unexpected, especially when studying seed characteristics, since the seeds are influenced by their development on the maternal plant and are surrounded by pod and testa tissue which are a previous generation. Breeders should be aware of this developmental process, especially when individual seed evaluations are made in early generations. Such maternal effects, however, last only one generation and have minimal consequence for a breeding program.

True cytoplasmic effects must be considered in breeding efforts, however. In a number of programs, including the one at Florida and the USDA-ARS program in Virginia, reciprocal crosses were made routinely as part of the artificial hybridization procedure for cultivar development. Often, after material has been selected for four to five generations using the pedigree method, progeny from a cross made in one direction will remain in the program while all the progeny from the reciprocal have been discarded. Although this phenomenon has been examined in detail, there is no clear *a priori* information that could be used to determine the appropriate direction of a cross for development of superior breeding lines. Whereas this evidence supports the need to make reciprocal crosses, the total number of crosses made in a breeding program is usually fixed because of resource limitations. Relative advantages of assessing progeny from crosses when a given parent is used both as a male and as a female must be weighed against the advantages of assessing completely different parent combinations.

The reciprocal cross differences previously cited have been the result of genetic contributions exclusively from the female parent. Paternal inheritance of glutamate oxaloacetate transaminase enzyme patterns was reported by Grieshammer and Wynne (1990b), and paternal inheritance of a shriveled seed trait also has been discovered (Jakkula *et al.*, 1993). Such a phenomenon is rare in the plant kingdom, and clear explanations for these results have not yet been proposed.

Artificial Hybridization. Techniques for artificial hybridization of peanut have varied little since originally reported in 1910 by van der Stok (Coffelt, 1989a). Artificial hybridization procedures currently in use have been summarized by Nigam *et al.* (1990). The crossing process is tedious, resulting in relatively few hybrid seed per unit time compared to many plant species. Some breeding procedures, such as recurrent selection and diallel selective mating, are used infrequently in peanut breeding because of the time required to make hybridizations. For the most part, however, the generation of new recombinants is not a limiting factor for cultivar development in peanut breeding programs. Rather, the substantial cost of time and labor for evaluation is the major constraint to the number of breeding lines that can be surveyed in most programs (Knauft *et al.*, 1987).

The study of peanut floral biology is useful not only for the development of efficient artificial hybridization procedures, but also for understanding cross-pollination mechanisms and their frequency. Absence of cross-pollination is important in maintaining homozygosity of lines in a breeding

program and during seed increase of cultivars for commercial production. Although most reports of outcrossing have indicated rates of 2% or less (summarized by Knauft *et al.*, 1992), more varied amounts of outcrossing have been reported. Levels of outcrossing in Virginia varied from 0 to 2.8% (Coffelt, 1989a), while rates in Florida varied from 1.0 to 8.1% (Knauft *et al.*, 1992). The variation in rates found in the latter study may be attributable to genetic differences in time of anther dehiscence. Breeding lines with over 20% outcrossing rates have been reported from mutation studies, although the researchers admitted that distinction between outcrossing and mutation was difficult (Dutta *et al.*, 1987). It is not known whether occurrence of high outcrossing rates in breeding lines is common. If rates can customarily approach 8%, outcrossing could be a considerable source of variability, especially in advanced breeding lines that are grown in close proximities in breeding nurseries. Recently, the Florida program expanded breeding work with lines containing white testae controlled by a single dominant gene (Knauft *et al.*, 1991a). Many breeding lines that had no history of spontaneous mutations for testa color began to show seed with white testa. Some of the seed were planted and were found to segregate, suggesting outcrossing rather than mechanical mixture. In some environments, the rate of outcrossing may be higher than anticipated. Since many breeding lines are phenotypically similar, outcrossing may have been occurring, but went undetected because segregants were not obviously distinct. It is unlikely that breeders can or should readily change procedures for development of breeding lines to decrease chances of outcrossing. Nevertheless, the higher rates of outcrossing may explain unexpected variability in late generation breeding lines.

Early Generation Testing. Early generation testing may be advantageous in reducing the amount of undesirable material in a breeding program by providing information for the discarding of entire crosses and allowing more resources to be used in evaluation of desirable germplasm. Iroume and Knauft (1987a) predicted, from heritability estimates, that early generation selection for crosses with superior yield and leaf spot resistance should be possible, but that selection of individual plants or plants within crosses would be inefficient. Halward *et al.* (1990) found early generation testing in a recurrent selection program was not effective in identifying source populations of breeding lines with high pod yield. Results from both investigations indicated that early generation testing may be appropriate for traits with high heritability, but not with traits of low heritability. Since traits with high heritability can be eliminated directly in the pedigree method, and through slight modifications of the bulk and single seed descent (SSD) methods, the advantage of early generation testing must be weighed against the costs and relative efficiency of other procedures.

Breeding Methods

Mass Selection. Mass selection is an old breeding procedure that may be used effectively in peanut cultivar development when selection can be made for traits with high narrow-sense heritability. Mass selection can be used to reduce the cost of maintaining variability in early generations, since minimal

input is required to advance generations and to cause desirable shifts in gene frequencies.

Several studies have examined the utility of mass selection in peanut breeding. In crosses between adapted and unadapted materials it was used to increase yield in intersubspecific crosses, but was less effective in intrasubspecific crosses, perhaps because of negative correlations between seed yield and meat content (Holley and Wynne, 1986). Knauft *et al.* (1993a) found the technique was not advantageous in developing high yielding, leaf spot-resistant lines, possibly because of negative correlations between yield and resistance. Patra *et al.* (1992b) used mass selection for pod yield/plant to advance breeding lines from the F_3 to the F_5 generation. They found the technique was successful in increasing mean pod yield, plant height, and harvest index in the F_5 lines. However, pod yield was associated with lower shelling percentage and smaller seed size. The consistent negative correlations between yield and other desired traits suggest the need to use some type of index selection if this procedure is used to combine desired traits.

An additional concern of using mass selection is that competition among genotypes may favor those types that are less productive when grown as pure lines. In one of the few studies of this type in peanut, it was shown that more competitive genotypes could predominate genetically mixed populations of peanut when grown over several seasons, although they were not the highest yielding pure lines (Knauft and Gorbet, 1991).

Pedigree Method. The pedigree method continues to be used frequently in peanut breeding programs. Little research has been published comparing the efficiency of this method in peanut with other procedures for self-pollinated crops. Given the high cost of this method compared to most other techniques, advantages likely exist for its persistent use. The unique biology of peanut may favor the use of the pedigree method. Because the economic yield of peanut is only visible after harvest, virtually all selection work occurs at that time. Observations of individual plants can be followed easily by selection of separate plants. When crossing programs are first established, it is possible to use diverse parents that provide segregants for highly heritable traits such as plant type, branching pattern, chlorophyll patterns, dwarf stature, pod size and shape, and testa color. Under such conditions, the pedigree method clearly could be used efficiently to eliminate unwanted material.

Branch and Hildebrand (1989) made pedigree selections in Zimbabwe and Georgia from the same segregating base population. However, they found that lines developed in each location exhibited significant genotype x environment interaction for pod yield and were generally unadapted to the other location. Branch *et al.* (1991) also examined a sequential method, making individual plant selections at one location and then sequentially cycling to a second and a third location for subsequent selection. In general, the sequential selections were equal to or better than pedigree selections made at a single location for yield and leaf spot resistance. However, unselected bulks developed with the single seed descent method generally performed as well or better than the sequential selections. While this

procedure may have potential for developing germplasm with wider adaptability, particularly for traits with high heritability, it requires considerable cooperation among breeding programs. Royalty-sharing differences among institutions and proposed exclusive releases of cultivars may limit the usefulness of this procedure in the future.

Backcross Method. The backcross method has been used sparingly in peanut breeding programs. The low number of seed produced from artificial hybridization in peanut make the backcross difficult to use, as does the limited number of economically important traits with simple inheritance (Wynne and Halward, 1989). The procedure has been used to incorporate the high oleic/low linoleic acid trait (controlled by two recessive genes) found by Norden *et al.* (1987) into breeding lines. In the Florida program, the original source of the trait, breeding line F435, has been used to cross to a component line of the Sunrunner cultivar, F519-9. The recurrent F519-9 parent was homozygous recessive for one of the two genes responsible for the trait, simplifying the backcrossing procedure. Evaluation of the trait was made more efficient by the development of a rapid technique to identify fatty acid composition from as little as 15 mg of tissue from individual seed (Zeile *et al.*, 1993a). This technique was used to identify segregates after self-pollination. Six backcrosses were made to generate breeding lines that contained agronomic, market grade, and processing characteristics similar to Sunrunner and the fatty acid composition of F435 (Knauft *et al.*, 1993b).

Single Seed Descent Method. The single seed descent method has received increased attention from breeders, a result of its potential utility in decreasing time to cultivar development and the lower cost associated with advancement of early generations. The procedure, as outlined by Brim (1966), has undergone modification in some programs. In Florida, the single seed descent method was used to advance generations in many crosses. Segregating material was planted at commercial densities, saving land and production inputs. A single pod, or sometimes two in early generations, was saved from each plant to maintain population variability while homozygosity was increased. Two field-grown generations were obtained each year at Gainesville, FL. Material planted in early April was harvested approximately 100 days after planting, and the most mature pods harvested. After drying and shelling, seed were planted in the field in late July. Seed dormancy was broken with an in-furrow application of a 0.01% solution of ethrel, applied at a rate of 2 L of solution/m of row. The July planting was harvested by the middle of November at Gainesville. Although the April-July-November sequence did not allow adequate maturation of a majority of seed on a plant, the earliest-set pods had sufficient physiological maturity to germinate at a high rate the following generation.

In Florida, intersubspecific crosses are not carried with the single seed descent method. Because segregants from such crosses frequently express undesirable, simply inherited characteristics such as dwarfism, chlorophyll deficiencies, and sparse branching (Dwivedi *et al.*, 1984), these plants are readily eliminated when using the pedigree method. The high plant densities used with the single seed descent procedure make it difficult for the breeder

to observe individual plants for some characteristics, such as sparse branching and dwarfism.

However, most intrasubspecific crosses, especially between parents with similar genetic backgrounds, are advanced through single seed descent. Although Halward and Wynne (1991) suggested that crosses between more closely related parents may more effectively be selected in early generations than genetically divergent parents, they based these conclusions on increased importance of additive gene effects. Rather than selecting for quantitatively inherited traits, the choice to include wider crosses in pedigree selection instead of single seed descent is based on the ability to remove simply inherited traits rapidly.

Single seed descent procedures have been proposed or used by several researchers. Anderson *et al.* (1990b) assessed plants in early generations from a diallel cross for multiple foliar pest resistance. They found high environmental variation limited usefulness of evaluation, but indicated such crosses with rapid movement to homozygosity by single seed descent could be used to improve chances of obtaining multiple pest resistance in peanut lines. In the previously cited study of sequential selection (Branch *et al.*, 1991), unselected bulks derived from single seed descent performed as well as selections made from this modification of the pedigree method. Despite these suggested advantages of the single seed descent procedure and its increased popularity as a peanut breeding tool, studies comparing its efficiency to that of other methods have not been published.

Recurrent Selection Method. Recurrent selection permits the incorporation of diversity in a breeding program for self-pollinated crops while providing opportunities for recombination. The added recombination improves the probability of uniting the various desirable traits into a single genotype and has the potential to be useful when many desired traits must be combined from separate sources. Recurrent selection based on the procedure of Compton (1968) was adapted to peanut by the breeding program at North Carolina State University (Wynne, 1976; Monteverde-Penso *et al.*, 1987). Three cycles of recurrent selection within a population derived from randomly crossing 40 elite virginia market-type peanuts had produced consistent gains in pod yield (Monteverde-Penso and Wynne, 1988). These gains were not correlated with specific fruit traits, allowing the selection of desired combinations of these characteristics with high pod yield.

After four cycles of selection within this population, Halward *et al.* (1990) found a decrease in genetic variation for pod yield with each cycle of selection, but an increase in the number of lines with pod yield equal to or greater than the check cultivar. Further evaluation of this material (Halward and Wynne, 1992) revealed that average improvement for pod yield and most other fruit traits measured had not progressed after the third cycle was reached. Although genetic variation had been reduced, significant genetic variability remained for all measured traits, indicating that further progress should be possible. The authors suggested that the lack of progress was the result of a low selection intensity of 40% used on the population.

In contrast to their previous studies using adapted genetic material, Guok

et al. (1986) studied the use of recurrent selection to improve both yield and leaf spot resistance in a population derived from an interspecific hybrid between *A. hypogaea* and *A. cardenasii* Krapov. and W. C. Gregory. After two cycles of recurrent selection they found improvement in fruit yield and many market grade characteristics. Halward *et al.* (1991b) examined the amount of genetic variability remaining in this population after the two cycles and found significant levels existed for 12 morphological traits and leaf spot resistance. They concluded that additional progress through selection could be made in this population for many economically important traits.

Complex Crosses. Another procedure proposed to break linkage blocks and recombine appropriate genes in a manner similar to that of recurrent selection is the use of complex crosses. Three-way crosses have been used in studies to broaden the initial genetic base of peanut germplasm (Arunachalam *et al.*, 1985). In the F_2 generation, plants were recovered that transgressed the better parent for yield components up to 370%. General combining ability of early growth stage characteristics was found most appropriate for identification of parents for complex crosses. Three-way crosses were found by Bandyopadhyay *et al.* (1985) to be superior to single crosses for improving physiological and yield components. A convergent hybridization scheme of crossing progressively more F_1 hybrids within cycles was conducted for four cycles in the development of a Coastal Plain Experiment Station germplasm population that contains segregating material from 16 parental lines with varying types of pest resistance (Branch and Holbrook, 1991). The authors proposed that this procedure would allow incorporation of characteristics from a large number of sources and provide potential for reducing linkage.

Few studies have compared progeny from the initial complex crosses with either single crosses or recurrent selection schemes. D. A. Knauft and R. N. Iroume (unpubl. data, 1987) compared F_5 lines derived from pedigree selection of single and double crosses made to incorporate high pod yield and late leaf spot resistance. They found no differences in pod yield or resistance between lines from the two types of crosses. However Dutta *et al.* (1986) found early generation intermating produced superior progeny for yield and yield components. It is expected that procedures for the development of complex traits would be appropriate for reducing linkage blocks. Low numbers of seed from artificial hybridization reduce the utility of this procedure in peanut.

Index Selection. One of the difficulties of selection in segregating generations is the need to identify plants with concurrent expression of many desirable characteristics. Index selection may allow improvement of selection efficiency in early generations. Iroume and Knauft (1987b) developed an index for simultaneous selection of pod yield and late leaf spot resistance and found the index to be a useful strategy. Bandyopadhyay *et al.* (1985) found a more complex index which included physiological components was more efficient for identifying superior genotypes than the index based on yield components alone. They did not compare their results to direct selection, however. Anderson *et al.* (1990a) recommended using principal component

analysis to examine components of multiple pest resistance in a 10-parent diallel.

Because low heritability limits effectiveness of direct selection for pod yield on an individual plant basis in early generations, many researchers have examined the correlation of more highly heritable characteristics with pod yield. If such traits can be identified, selection based on their expression would be expected to improve yield as well, and also improve efficiency as compared to direct pod yield selection. Vegetative characteristics in particular would be useful traits for indirect selection since they could be evaluated prior to harvest. A summary of many correlation, or character association, studies has been published (Reddy, 1988). Vinod Prabhu *et al.* (1990) found measurement of nitrogen percentage and leaf area explained 75% of the variation in pod yield among 17 genetically diverse lines. They concluded that these characteristics could be used for indirect selection for yield to remove poorer genotypes as a first step in improving selection efficiency. Of 16 vegetative and reproductive characteristics examined in a large study by Nigam *et al.* (1984), only pods/plant, pod weight, and seed/plant would be effective as characteristics that could be indirectly selected for yield. Nagabhushanam and Prasad (1992b) and Nagabhushanam *et al.* (1992) found several traits, especially canopy diameter/plant at 60 days, to be stable individual plant characteristics highly correlated with pod yield. They suggested that choosing plants based on canopy diameter at 60 days, in addition to the selection criteria of pod number and yield/plant, would improve efficiency of selection. Manoharan *et al.* (1990) found pod number, individual pod weight, and dry matter production were less influenced by the environment than was pod yield. Given the high correlations of these traits with yield, they proposed their use in selection schemes to improve efficiency.

Genotype-by-Environment Interactions

One of the most important aspects of cultivar development procedures is the identification of lines that consistently express superior performance of important characteristics when grown in different environments. However, genotype-by-environment (g x e) interactions for yield and market grade are frequently found in peanut (Dashiell *et al.*, 1982; Shorter and Hammons, 1985; Knauft *et al.*, 1986; Norden *et al.*, 1986; Anderson *et al.*, 1989; Coffelt *et al.*, 1993; Raut *et al.*, 1993;). Assessment of stability for production and market grade requires testing in multiple years and locations, increasing the cost of cultivar development and delaying time to release. Cooperation among breeders for regional and national testing of advanced lines is an efficient method for stretching limited budgets. Elimination of breeding programs and varying policies of royalty assessment and distribution is restricting such cooperation.

Methods of Evaluation. Several procedures have been suggested for improving the efficiency with which interactions between genotypes and environment are measured. Shorter and Hammons (1985) used pattern analysis to determine causes of g x e interactions. They found interactions were specifically the result of maturity being confounded with harvesting

procedures, but found no evidence that genotypes derived from different breeding programs had different environmental responses. Although various stability parameters can be used to measure genotype response to environment, Anderson *et al.* (1989) found distance parameters and regression analyses gave more precise information than other stability parameters used. Shorter and Butler (1986) found several types of moving mean covariance adjustments could be used to reduce error variances and improve selection efficiency in breeding nurseries through reduction in g x e effects. Coffelt *et al.* (1993) found differences in estimates of genetic variance components affected g x e interactions. They indicated crosses producing superior yield and market grade could be identified at a single location, but that multiple years and locations were required to select the best performing lines within crosses.

Market Grade Stability. While pod yield must clearly be evaluated in many environments prior to cultivar release, several components of market grade may be more stable. Although market grade shows g x e interactions (Knauft *et al.*, 1986; Anderson *et al.*, 1989), rank correlations for shelling percentage and seed size were highly consistent in different environments (Knauft and Gorbet, 1993). Thus, identification of superior lines for these traits could be made with minimum testing. If shelling percentage and seed size are important components in potential cultivars, the authors indicated lines could be discarded based on relative expression of these traits early in the evaluation process of a breeding program. This would allow resources to be used more efficiently for measurement of pod yield. As expected, wide variation in rank correlations occurred for this trait.

Use of Genetic Mixtures. Genetic variability within cultivars may be an important aspect of yield stability. Because self-pollinated cultivars frequently are composed of similar genotypes, this contribution of stability is generally limited. It is theoretically possible to mimic some genetic advantages of cross-pollinated species by purposely mixing lines of self-pollinated crops (Jensen, 1988). A number of peanut cultivars, particularly those from the Florida breeding program, including the widely grown Florunner and Florigiant cultivars, have been developed through blending of sister lines (Knauft *et al.*, 1987). In theory, the genetic variation within these mixtures can give a buffering capacity to biotic and abiotic stresses, since the component genotypes may have different responses to stress. Blends from the Florida program were originally developed based on phenotypic uniformity among component lines without determining the relative stability of mixtures compared to individual lines.

Several studies have been conducted to determine whether mixtures made without *a priori* knowledge of stability performance would be either higher yielding or more stable than component lines. These studies have generally shown little advantage of mixtures over the best component line for yield and/or grade stability, especially when lines were combined without prior testing for stability performance. Schilling *et al.* (1983) found that composing blends from sibling lines rather than diverse pure lines was most feasible for circumventing g x e interactions for pod yield, particularly when

genetic variability could be maintained among the sibling lines. They noted, however, that pure lines could be identified that were as stable as multilines. Norden *et al.* (1986) found no significant differences in average pod yield or market grade characteristics when comparing four mixtures and the four component lines making up each mixture. Although stability of the different lines varied, stability generally was not improved by blending breeding lines. Knauft *et al.* (1986) found no increase in stability of market grade characteristics when comparing four multiline cultivars with two single-genotype breeding lines. Rattunde *et al.* (1988) composed various proportions of two peanut genotypes differing in growth habit and found no mixture with higher yield than the best component line. Knauft and Gorbet (1991) found the best component line was superior to a mixture of five lines for pod yield and market grade characteristics. Patra *et al.* (1992a) obtained an advantage when pod yield of a mixture was compared to the average yield of its component lines, but that individual lines had higher yield and were more stable than the mixture. One advantage pointed out by Norden *et al.* (1986) is that composing cultivars with several sister lines would allow the modification of the genetic make-up of a cultivar based on additional evidence of yield or grade stability gathered after the release decision is made. Two multiline cultivars, Florunner and Florigiant, have been highly durable. Florigiant was widely grown in the Virginia-Carolina region of the U.S. for over 20 years; and Florunner, released in 1969 (Isleib and Wynne, 1992), has been the most popular runner cultivar in the U.S. for nearly 25 years. It is possible that their consistent performance is the result of genetic diversity within the lines resulting from a combination of their component lines and early generation selection.

These studies do not indicate that mixtures are inherently less stable or lower yielding than pure lines, but that mixtures may need to be constituted after testing various component lines for yield superiority and stability rather than constituting mixtures based on phenotypic similarity alone. Unfortunately, testing of various combinations of lines for stability will add considerable cost to cultivar development. Any advantages of mixtures also must be balanced with the price of maintaining the separate lines in a breeding program and the constant need for reconstitution for production of certified seed. For example, 18 distinct breeding lines must be maintained to constitute the four cultivars Florigiant, Florunner, Sunrunner, and Early Bunch developed by the Florida program.

Traits Associated with Stability. Efficiency of selecting breeding lines with stable yield or market grade expression could be improved by the identification of phenotypic traits associated with improved stability. Several researchers have examined characteristics to determine their relationship to early maturity. Knauft *et al.* (1990) identified rapid early vegetative growth and subsequent high but not absolute partitioning of photosynthate to pods as important characteristics in more stable genotypes. Nagabhushanam and Prasad (1992a) found intermediate canopy types gave more stable yield than either upright or spreading types.

Maturity. Many additional factors have been associated with yield

stability, including time to maturity, response to temperature and photoperiod, and reactions to different types of stresses (Nigam *et al.*, 1991). Genetic control of maturity is difficult to quantify, primarily because of the inherent challenge of defining maturity of the indeterminant, nonsenescent peanut plant (Kvien and Ozias-Akins, 1991). The results of genetic studies to ascertain the inheritance of maturity often vary depending on the method of maturity measurement used in the research. Holbrook *et al.* (1989) defined maturity with the hull-scrape method (Johnson, 1987). Based on high broad-sense heritability estimates from a cross between parents with large maturity differences, they concluded that the hull-scrape method could be used effectively to manipulate peanut germplasm for this trait. Khalfaoui (1990) found maturity to be conditioned by few genetic factors, but highly influenced by the environment. Time to emergence, time from emergence to flowering, number of flowers, and proportion of mature pods (based on internal pod color) were used in this study and had low correlations with each other. These conclusions are corroborated by the effective use of pedigree selection for development of early maturing peanut cultivars. Using phenotypic traits of leaf yellowing, external and internal pod maturity traits, seed size and shelling percentage, Gorbet *et al.* (1992) developed the Marc I cultivar, which has a maturity 10 days earlier than standard runner market-type cultivars. A second early maturing cultivar, Andru 93, was developed using this procedure (Gorbet and Knauft, 1994), and both VA 81B and VA 93B were produced in a similar manner (Coffelt *et al.*, 1982,1994).

Studies have been conducted with valencia peanuts to determine the utility of selection for early emergence and maturity when grown in the cool environment of Ontario, Canada (Michaels, 1988). Selection of early maturing peanuts based on percentage emergence and sound mature seed yield in this short-season environment was difficult. He found maturity based on these parameters to be complexly inherited.

Another procedure used for the development of early maturing peanut lines is the concept of thermal time, calculated on a base temperature above which peanut development occurs and a maximum temperature above which no development takes place. Vasudeva Rao *et al.* (1992) used a mean base temperature of 10 C with no maximum temperature to calculate thermal time. Rather than use a calendar basis, they harvested peanut lines after a minimum cumulative thermal time had been reached and then evaluated pod yield. The procedure was successful for identifing lines with higher yield at early harvest dates. Nigam *et al.* (1988) found time of seedling emergence to first flowering was influenced predominantly by additive genetic variance, but the relationship between first flowering and maturity was not discussed. Mutation breeding for early maturity as defined by yield at early harvest may be possible (Mouli and Kale, 1989).

Tolerance to Abiotic Stress. The ability to tolerate environmental extremes also may be an important component of cultivar development for stable yields. Large genetic differences in rate and percentage of germination have been identified for a range of temperatures (Mohamed *et al.*, 1988a), and other researchers have found variation for these traits in cool temperatures

(Bhagat *et al.*, 1988). In many parts of the world, lower temperatures affect not only germination, but early season growth as well. Although these results indicate that it should be possible to breed for germination in cool temperatures, conflicting results have been reported for genetic variation of early growth in cool temperatures. Mohamed *et al.* (1988b) found little variation in seedling emergence and leaf growth at a range of temperatures, while Bhagat *et al.* (1988) found genetic differences in a number of growth measurements made at cool temperatures.

Genetic variability also exists in peanut for response to poor soil conditions. Branch and Gascho (1985) were able to identify tolerance, based on total sound mature kernel yield, to low soil fertility among 24 cultivars. They found the response was not related to any single soil or tissue nutrient. Kesava Rao *et al.* (1990) found genetic variation among nine peanut cultivars for phosphate mobilization, and a substantial body of information has been published on variation for nitrogen fixation activity and its inheritance (for example, Arrendell *et al.*, 1988, 1989; Phillips *et al.*, 1989; and Chapter 8).

The poor water-holding capacity of most peanut soils makes tolerance to drought stress an important objective in breeding programs. Several studies have identified genetic differences in drought tolerance (Ravindra *et al.*, 1990; Branch and Kvien, 1992b). Other aspects of response to temperature extremes, photoperiod, and drought are discussed by Williams and Boote (see Chapter 9).

Tolerance to Interplant Competition. Yield stability in peanut may include other characteristics besides desired responses to biotic and abiotic stresses. The ability to tolerate interplant competition also may be a component of stability. This trait has been examined in a number of contexts. Many studies have been conducted to examine intragenic competition based on yield response to population differences. Most of these studies have shown little genotypic response from differences in intrarow competition within pure lines of similar plant type for yield, grade, or net value (e.g., Jaafar and Gardner, 1988; Mozingo and Steele, 1989). This indicates that, within plant types, specific development of cultivars for low or high populations would not be effective. It also suggests the procedures used to select within segregating populations in breeding nurseries should be effective for identifying types with desirable traits at commercial spacings. Similar results were found while examining relative responses of pure lines to leaf spot pressure at different levels of interplant competition (Knauft and Gorbet, 1989c).

Several studies have been conducted examining genetic differences in response to interspecific competition. In some cases, such as competition with weeds, it is desirable to identify peanut genotypes with the ability to compete effectively. Fiebig *et al.* (1991) identified a peanut genotype less affected by weed competition (from *Xanthium strumarium* L.) than other lines, suggesting the possibility of developing cultivars with reduced yield losses from weeds. In other instances, such as cases where peanut is intercropped, the ability to produce pod yields effectively without reducing yield of companion crops is desirable. Several studies on genotype response

of peanut to intercropping with cereals have shown little genotype interaction with intercropping, suggesting that the highest yielding peanut line grown in pure stands also would be the most productive types in intercrop systems (Knauft, 1984; Ndunguru and Williams, 1993).

The preceding discussions have focused on the development of peanut genotypes with adaptable and stable performance based on the *a priori* development of improved performance for specific characteristics. Few studies have been published on the development of peanut cultivars for high productivity in environments with low input attributable to a number of causes. For development of these types of cultivars to be feasible, genetic variance must be greater in stress environments than in nonstress environments, a situation that may not be common (Rosielle and Hamblin, 1981). Using four levels of input, Knauft and Gorbet (1989a) found different genetic responses in pod yield and found genetic variance was not related to the level of stress in the set of conditions in their experiment. They identified a genotype with high and stable productivity each for the high and low input environments. They suggested using modified stability analysis and economic analyses of net benefit to assist in identifying genotypes with productivity in specific high or low stress conditions. They cautioned that a portion of the response in their study may have been due to greater yield reductions in the stress environments from leaf spot pressure. As discussed in other chapters in this volume, pest resistance is an important aspect in the development of stable cultivars.

Marshall (1991) has emphasized the need for breeders to continue a breeding approach, designated "defect elimination," that identifies specific constraints that either limit yield or are major causes of yield instability. The development of resistance or tolerance to the major constraints may provide yield stability. It was suggested that this approach will continue to be relevant, in spite of alternate strategies identifying morphological and physiological traits that could directly contribute to higher yield and/or yield stability.

CULTIVAR RELEASES

Until recently, the majority of peanut hectarage in the U.S. was planted to cultivars made up of several component lines released to peanut growers by public institutions that set seed prices at levels consistent with the cost of seed production. The cultivar release process has changed in recent years. The last cultivar released as a composite was Southern Runner, which was composed of three F_6 sister lines (Gorbet *et al.*, 1987). Subsequent cultivars have been individual breeding lines. Ironically, the use of component lines has allowed the successful release of two cultivars from a composited breeding line that would have otherwise been too variable to release. In the Florida program, UF79308 was agronomically tested for several years. Despite superior yield and early maturity, it was found to contain excess variability for seed size. Because the component lines had been maintained separately, they could be tested individually. Two distinct single line

cultivars, Marc I and Andru 93, have been released from the original UF79308 (Gorbet *et al.*, 1992).

Many cultivars are available to peanut growers today. This is the result of greater emphasis placed on cultivar development by public institutions in the late 1980s and early 1990s and the addition of breeding efforts by a private company, AgraTech Seeds, Inc. The elimination of the programs at the University of Florida in Gainesville, New Mexico State University, and the USDA-ARS programs at Stillwater, OK and Suffolk, VA is likely to result in an overall reduction in cultivar development activity in the U.S.

While it is obvious that private companies require royalties for corporate profits, public institutions increasingly are imposing fees to assist in support of program operation. The effect of these changes on peanut cultivar development has been mixed. The potential to provide sufficient resources for development of improved cultivars increases with adequate funding. With added financial resources, programs will have capabilities to provide improved cultivars that can maintain or improve the economic status of peanut growers, shellers, and manufacturers of peanut products. On the other hand, some fee structures incorporate financial benefit to individual research programs and frequently personal benefit to the breeder. This has resulted in reduced exchange of enhanced germplasm and genetic information, and makes it less descriable for grower organizations to support breeding efforts.

GENETIC GAINS

Peanut breeding over the last 40 years in the U.S. has been successful in developing improved cultivars. Several studies conducted recently have examined genetic gains in pod yield from breeding activities and have identified the physiological processes affected by these efforts. An understanding of the basis for yield improvement in more recently released cultivars may be beneficial in the development of more efficient selection criteria for improved pod yield. Mozingo *et al.* (1987) found a yield increase of 18.5% attributable to genetic improvement in virginia market-type cultivars between 1944 and 1987. The yearly average yield increase of 14.7 kg/ha occurred largely from increased yield potential of cultivars released prior to 1980. More recent efforts to develop pest resistance and acceptable quality in peanut cultivars have been successful, but have not combined these traits with genes conditioning a continued increase in yield potential. Yields have been maintained while developing earlier maturity and improved blanchability and storage (Mozingo *et al.*, 1988). Seaton *et al.* (1992) and Coffelt *et al.* (1989) examined reproductive efficiency in 14 virginia market-type cultivars released since 1944. They found the later-released cultivars had greater partitioning of photosynthate to pods than earlier cultivars, and that later cultivars also produced more flowers. Wells *et al.* (1991) further defined the relationship between breeding efforts and physiological differences by examining yield and reproductive characteristics. They examined differences among cultivars based on the number of breeding cycles from introduced or

land race varieties. They found yield increases of 30 g/m^2 per breeding cycle. Increased breeding efforts, as defined by further cycles, gave plants with smaller vegetative mass, greater partitioning of photosynthate to pods, and earlier initiation of reproductive development. Similar studies with recently released runner market-type cultivars have not been made, although Knauft and Gorbet (1990) conducted growth analysis studies with leaf spot-resistant runner germplasm. They found lines with higher yield potential had greater partitioning of photosynthate to pods, although the high-yielding resistant lines had later initiation of pod formation than high-yielding susceptible material.

There are obvious limits to the trends of smaller vegetative mass, greater partitioning of photosynthate, and earlier initiation of reproductive development. Decreasing vegetative mass will, at some point, result in an insufficient source of photosynthate to fill pods. The smaller vegetative mass also may be less competitive with weeds and less able to sustain productivity upon defoliation from insects or diseases. High partitioning of photosynthate to pods will result in weaker vegetative systems, making the plant less competitive and more susceptible to stress. Beyond a certain point, earlier maturity will result in smaller plants that are less capable of responding to stress. With the exception of stress caused by a short growing season due to temperature or moisture limitations, other types of stress may be more readily addressed by maintaining some level of vegetative growth and development during pod fill.

INDUCED MUTATIONS

Cultivated peanut germplasm shows limited genetic variability for many important characteristics. This has led to the exploration of different methods for improving variability, including induced mutations. Mouli *et al.* (1989) summarized recent work on induced mutations in peanut. Many morphological changes have been observed after mutagenesis that may have value in genetic studies. These include variations in chlorophyll content, branching habit, leaflet morphology, flowering pattern, maturity, and pod and seed morphology (Mouli *et al.*, 1989). Thirty-three peanut cultivars have been released worldwide with the help of induced mutations (Anonymous, 1991). Of the 33 cultivars, 20 were developed in China. Most were released to provide improved seed size, earliness, or better yield. Nineteen cultivars were released directly after selection of mutagenized seed, and the remaining 14 were developed after selection from crossing mutagenesis-derived lines with other material. Given the stringent market requirements in the U.S. for peanut cultivars, it is not likely that the random genetic change caused by induced mutations directly will be used to produce released cultivars. However, changes in seed composition, such as oil, protein, or carbohydrate content and composition, may be amenable to modification by induced mutations. Given the small variation normally found in peanut for content of these components, induced mutations may broaden the range of expression

of these traits. For example, Gadgil and Mitra (1983) doubled the sucrose content of a cultivar with X- and gamma-ray induced mutations. Slight increases in oil content have also been induced (Sharma *et al.*, 1981). Induced mutations have been used to develop many mutations in fatty acid composition in plants such as *Arabidopsis* (Lemieux *et al.*, 1990). Given the range of fatty acid composition found in peanut (Norden *et al.*, 1987), mutation induction may be useful in manipulating decreases in total saturated fats, increases in stearic acid content, or decreases in long chain fatty acids. Mutations have also been induced for leaf spot resistance in peanut (Soriano, 1988).

TECHNIQUES TO EXPAND THE AVAILABLE GERMPLASM FOR PEANUT IMPROVEMENT

Additional techniques are available to expand the range of characteristics and their expression in peanut. Many *Arachis* species possess traits that would be desirable additions to *A. hypogaea* germplasm. However, most *Arachis* species are either cross-incompatible with cultivated peanut or cross with low efficiency. One method that has allowed the incorporation of some genetic material from the wild species into peanut is the use of embryo rescue following interspecific artificial hybridization. Rescue of hybrid embryos from crosses between species of *Arachis* could be a useful supplement to conventional breeding even though additional barriers to the utilization of hybrids (ploidy level, infertility) may be encountered (Stalker and Moss, 1987 and Chapter 2).

Embryo Culture

A distinct but under-utilized advantage of embryo culture is its application to the rescue of *Arachis* hybrids that can be obtained conventionally, but at low frequency, especially with certain genotype combinations (Ozias-Akins *et al.*, 1992c). This technically simple procedure could greatly increase the efficiency of an interspecific crossing program targeted at compatible crosses, as well as provide a more controllable (*in vitro*) environment for colchicine doubling of chromosomes and rapid estimation of ploidy levels (Singsit and Ozias-Akins, 1992).

Historically, plant embryos have been separated from maternal tissues at the time of culture (Collins and Grosser, 1984) or cultured *in ovulo* (Rangan, 1984). Rarely have excised embryos less developed than the heart-shaped stage been successfully cultured (Raghavan, 1980; Collins *et al.*, 1984). Many of the desirable crosses between *Arachis* spp. result in small aborted proembryos (Halward and Stalker, 1987b). The unique reproductive biology of peanut adds an additional complicating factor to the rescue of pre-cotyledonary stage embryos: the peanut flower is an aerial structure but the fruit is hypogeal (Smith, 1950). Specific developmental and physiological processes are associated with the transition from aerial to subterranean

growth (Thompson *et al.*, 1985, 1992; Shushu and Cutter, 1990). Unlike most embryos, the peanut proembryo enters a period of quiescence after reaching the eight-celled stage (Pattee and Mohapatra, 1987). The conditions required for release of the proembryo from its quiescent state are not fully understood. Clearly, embryo culture with existing methods may not be sufficient to overcome the apparently irreversible quiescence of some hybrid *Arachis* proembryos (Pattee *et al.*, 1988). Avoidance of events in part correlated with quiescence [i.e., eliminating gynophore meristem activity (Moss and Stalker, 1987) or alleviating developmental asynchrony resulting from genotype interactions (Halward and Stalker, 1987a,b; Pattee and Stalker, 1992)] has been the approach taken in several recent studies (Moss *et al.*, 1988; Pattee *et al.*, 1988; Stalker and Eweda, 1988). Zygotes existing one day post-pollination have been encouraged to develop to the globular embryo stage by culturing 1-mm long peg tips on a nutrient medium optimized for growth regulator composition (Rau *et al.*, 1992). The transition from globular to heart-shaped embryos has not been obtained in cultures of meristem-less peg tips. Older (but still aerial), 12-to-15 mm-long pegs harvested after the initiation of the geotropic response could be cultured on a medium that allowed pod and presumably embryo development (Ziv and Zamski, 1975; Ziv and Sager, 1984). Culture of young pegs from cultivated genotypes has been successfully grown to plants (Feng *et al.*, 1994), but culture of hybrid embryos in this manner remains to be tested. Another technique that may improve the success of interspecific hybrid embryo culture is the combination of hormone treatments at the time of pollination with *in ovulo* embryo culture. By this method, Sastri and Moss (1982) were able to rescue a limited number of intersectional hybrid embryos and plants.

Recent progress with embryo culture of other species has been made by modifying media, culture technique, or explant. Campenot *et al.* (1992) were able to isolate embryo sacs of *Zea mays* L. at 1 day post-pollination by microdissection. The embryo sacs could not be completely separated from nucellar tissue at the time of culture, thus it is likely that the nucellar cells functioned as nurse cells. Forty percent of the cultured zygotes developed to maturity and germinated on a modified Murashige and Skoog (1962) medium containing 0.1 mg/L benzylaminopurine (BAP). Growth regulator requirements for optimal response of cultured embryos may be stage and species specific. No growth regulators were necessary for development of early globular embryos of *Brassica juncea* L. to maturity *in vitro* (Liu *et al.*, 1993). Key modifications that contributed to the success with *Brassica* were composition of the culture medium, a bilayered medium designed gradually to reduce osmotic pressure, and encapsulation of the embryo in agarose medium.

Anther and Ovary Culture

In a number of plant species, anther and ovary cultures have been used to produce haploids. In addition to genetic studies and bridges for introgression of wild germplasm, doubled haploids would provide rapid development of homozygous breeding lines. Unfortunately, minimal progress has been

made with anther culture in peanut to this point. Criteria for selecting anthers at the proper developmental stage for *in vitro* culture have been established based on floral bud size and anther color, although genotype and plant age affected absolute bud size (Sudhakar and Moss, 1990). Anthers containing early uninucleate microspores gave the highest response for microspore mitosis (production of four-nucleate cells) on the optimal medium (Willcox *et al.*, 1991). Development beyond the four-nucleate stage was not observed. Although embryos from pollen reportedly were produced by Bajaj *et al.* (1981), no documentation of haploid peanut plants was presented. Limiting factors for anther culture in *Arachis* are the difficulty in obtaining large numbers of anthers at the proper developmental stage; a low frequency of pollen embryo formation; and no documented differentiation of the pollen embryos into plants. Induction of *in vitro* flowering from cotyledons of peanut (Narasimhulu and Reddy, 1984) might be one method to produce flowers under controlled conditions for anther culture. Certain genotypes produced as many as 28 flower buds per explant from 20% of the cultured, deembryonated cotyledons.

Tissue Culture

Tissue culture also can be used for the study of metabolic processes in suspension cultures as well as *in vitro* plant regeneration, which is an essential component of somatic hybridization, somaclonal variation, and genetic transformation. The tissue culture literature on peanut prior to 1982 has been reviewed (Ketring *et al.*, 1982; Bajaj, 1984), thus the present summary will highlight primarily subsequent published information. Results from most early tissue culture studies on *A. hypogaea* indicated that undifferentiated callus could be induced from a variety of explants (Narasimhulu and Reddy, 1983), but shoot formation from serially subcultured material was either lacking or sporadic. When shoot formation did occur, it usually was confined to the primary cultures (Mroginski *et al.*, 1981; Pittman *et al.*, 1983; Atreya *et al.*, 1984; Bhatia *et al.*, 1985), not subcultures, suggesting that preexisting or explant-derived primordia were the underlying source of these shoots. Even now, there is no clearly documented example of *de novo* shoot formation from totally undifferentiated tissues of peanut. The more recently developed *in vitro* methods for shoot regeneration from peanut have relied on the intrinsic competency of the cultured explant tissues for embryogenesis or organogenesis. The difficulty with inducing undifferentiated tissues to regenerate will be a hindrance to the application of single cell methods, such as protoplast culture, to genetic modification within the species.

A parallel perhaps can be drawn between peanut and cereals with respect to differentiation from undifferentiated tissues. In cereal tissue cultures successful plant regeneration from protoplast culture resulted only when the source tissue (i.e., embryogenic suspension, young leaf bases) was already competent for plant regeneration (Ozias-Akins and Lörz, 1984; Vasil and Vasil, 1992; Gupta and Pattanayak, 1993). The same phenomenon has been observed with more recent efforts to develop protoplast culture methods for

peanut. The first protoplast culture attempts in peanut used expanded leaves from *in vitro*-grown plants as a source of protoplasts (Oelck *et al.*, 1982). Protoplasts could be isolated in substantial yields and cultured to the callus stage (Oelck *et al.*, 1982; Mhatre *et al.*, 1985; Rugman and Cocking, 1985). No shoots were regenerated in any of these studies. Although conditions for induction of shoots possibly could be determined empirically, the probability is low because shoots have never been induced from fully expanded leaf blades of peanut. Leaves of *A. hypogaea* appear to be competent for shoot formation only for a short period during development. Young leaflets 2- to 5-mm long were capable of producing small numbers of shoots at a high frequency during the primary culture stage (Mroginski *et al.*, 1981; Pittman *et al.*, 1983). No shoot regeneration was obtained from fully expanded leaflets. For most genotypes, the frequency of shoot formation from young leaflets has been low (Seitz *et al.*, 1987; McKently *et al.*, 1991; Clemente *et al.*, 1992) and suspected to be dependent on environmental factors (Seitz *et al.*, 1987). Valencia-type peanuts respond with higher frequencies of shoot bud formation than virginia- or spanish-botanical types when a specific explant (petiolule with blade attached) is cultured (Cheng *et al.*, 1992). Buds developed only from the petiolule tissue and not from the expanded leaf blade. One highly regenerable tissue is the immature cotyledon of peanut (Ozias-Akins, 1989). Li *et al.* (1995) have exploited this characteristic by isolating protoplasts directly from immature peanut cotyledons. Cotyledon protoplasts can divide when cultured with nurse cells, and somatic embryos have been produced from protoplast-derived colonies. The frequency of embryo formation and conversion currently is too low, however, for the technique to be routinely useful for genetic transformation.

Other *Arachis* species exhibit a greater capacity for regeneration from mature tissues than *A. hypogaea*. *Arachis villosulicarpa* Hoehne readily produces shoots from cultured expanded leaves (Dunbar and Pittman, 1992) and from roots (Pittman *et al.*, 1984). Suspension cultures initiated from mature leaf callus of *A. paraguariensis* (Chodat & Hassl.) remained totipotent for at least 2 months (Li *et al.*, 1993) as did suspension cultures initiated from anthers of the same species (Still *et al.*, 1987). Li *et al.* (1993) have again exploited the totipotent condition of *A. paraguariensis* suspension cultures to regenerate plants from protoplasts of this species. The regeneration potential of protoplasts from wild species might be expressed in somatic hybrid cells derived from fusions with less competent protoplasts from *A. hypogaea*. Such a phenomenon has been observed in *Citrus* somatic hybrids (Grosser and Gmitter, 1990).

The recalcitrance of peanut for plant regeneration is not surprising given the previous example of soybean, another grain legume. Methods for routine plant regeneration from soybean cultures evolved over several years into reproducible protocols for sustaining embryogenic (Ranch *et al.*, 1985) and organogenic (Wright *et al.*, 1986) developmental programs. Considerable refinement of these selected protocols has continued to reduce genotype specificity and improve conditions for genetic transformation. Progress in peanut regeneration and transformation has lagged behind soybean, likely

reflecting the relative importance of the crops and total research effort. Nevertheless, significant progress has been made over the past 5 years in development of regeneration and gene transfer protocols for peanut.

Plant regeneration from tissue cultures of peanut can occur through embryogenesis or organogenesis. The primary utility of tissue culture is as a tool for studying metabolism and development or generating genetic variation. In most cases, the desirable end-product is a plant. Shoots grow readily from excised shoot tips cultured under suitable conditions (Kartha *et al.*, 1981; Chen *et al.*, 1990). Shoot-tip culture has been useful for virus elimination in peanut, but only when combined with thermal treatment and an anti-viral compound (Chen and Sherwood, 1991). During *de novo* organogenesis, shoot meristems are initiated independently of root meristems (Haccius, 1978). These shoots usually can be excised individually and subsequently rooted. Somatic embryogenesis occurs when bipolar (i.e., shoot and root pole) structures are initiated and follow a developmental pathway similar to zygotic embryos. In peanut, both developmental programs apparently are initiated only in previously determined tissues and not in undifferentiated tissues; however, long-term, organized cultures of both morphogenetic types can be maintained (Fig. 1 in McKently *et al.*, 1990; Durham and Parrott, 1992; Ozias-Akins *et al.*, 1992b).

Prolific organogenesis can be obtained from seed parts, most generally the cotyledons or tissues surrounding and including the cotyledonary node. A combination of a-naphthaleneacetic acid (NAA) or indoleacetic acid (IAA) with BAP or kinetin or cytokinins alone in a defined culture medium (Narasimhulu and Reddy, 1983; Atreya *et al.*, 1984; McKently *et al.*, 1990; Daimon and Mii, 1991) or only water (Bhatia *et al.*, 1985) is sufficient to induce shoot formation. Auxins typically stimulate callus formation whereas cytokinins support continual multiplication of shoot meristems. A high level of BAP (25 mg/L) was found to be optimal for shoot proliferation in several *A. hypogaea* genotypes (McKently *et al.*, 1990). Rooting of shoots generally occurs on basal medium or medium supplemented with NAA.

Although structures suggestive of somatic embryos were observed in cultures initiated from cotyledonary nodes (Banerjee *et al.*, 1988) and young leaves (Pittman *et al.*, 1983), conversion of the putative somatic embryos to plants was not shown. A variety of auxins has been observed to elicit somatic embryogenesis when applied to immature cotyledons (Ozias-Akins, 1989), immature embryo axes (Hazra *et al.*, 1989), whole immature embryos (Sellars *et al.*, 1990), mature embryo axes (McKently, 1991), or young leaves (Baker and Wetzstein, 1992). Exogenous auxin type (NAA, 2,4-diclorophenoxyacetic acid, picloram, 2,4,5-trichlorophenoxyacteic acid) can influence embryogenesis quantitatively and qualitatively (Hazra *et al.*, 1989; Ozias-Akins, 1989; McKently, 1991). Thidiazuron, a growth regulator that normally invokes cytokinin-induced responses (Lu, 1993), also can promote somatic embryogenesis from intact seedlings (Saxena *et al.*, 1992) or seedling explants (Gill and Saxena, 1992) of peanut. All genotypes tested under inductive culture conditions have been competent for the embryogenic response, although genotypes differed quantitatively in the frequency and magnitude

of embryogenesis (Sellars *et al.*, 1990; Ozias-Akins *et al.*, 1992a). Regeneration of plants from peanut somatic embryos can occur through embryo conversion (germination and development of shoot and root) or multiplication of meristems at the shoot pole (Ozias-Akins *et al.*, 1992b). As demonstrated for soybean (Buchheim *et al.*, 1989), conversion frequency of peanut somatic embryos is correlated with embryo morphology (Wetzstein and Baker, 1993) and can be increased by dessication treatment of mature somatic embryos (Durham and Parrott, 1992).

Genetic Transformation

For designing a genetic transformation system, the most important considerations are the recipient tissue (Is it permissive for transformation? Can plants be recovered?) plus the DNA vector and delivery system (Will the vector DNA gain access to the permissive cells? Does the DNA contain a suitable marker gene—either selectable marker or reporter gene—that can assist in recovery of stably transformed cells/tissues/organs/plants?). Both embryogenic and organogenic cultures of peanut should be amenable to transformation either via *Agrobacterium* (Angenon and Van Montagu, 1992) or direct DNA methods suitable for DNA introduction through intact cell walls (Weissinger, 1992). Peanut tissues are susceptible to infection by wild-type strains of *Agrobacterium rhizogenes* (Mugnier, 1988) and *A. tumefaciens* (Lacorte *et al.*, 1991; Mansur *et al.*, 1993) producing roots or tumors, respectively, although strain/genotype interactions have been observed. Disarmed *Agrobacterium* strains containing a binary vector with the reporter gene, β-glucuronidase (*gus*), and a selectable marker gene, neomycin phosphotransferase (*npt*II), affect stable transformation of peanut hypocotyl segments. Kanamycin-resistant, GUS-positive callus has been selected, but no plants have been regenerated (Franklin *et al.*, 1993). A functional peanut stripe virus coat protein gene also has been stably transformed into peanut hypocotyl callus via co-cultivation with *A. tumefaciens* (Franklin *et al.*, 1993). Expression of the gene has been detected by immunological techniques. No plant regeneration has been achieved from these *Agrobacterium*-transformed tissues. Targeting the *Agrobacterium* transformation event to cells competent for regeneration is the essential remaining step. Preliminary evidence suggests that peanut embryo axes co-cultivated with *Agrobacterium* have been stably transformed (McKently *et al.*, 1993).

Direct DNA delivery is an alternative to *Agrobacterium*-mediated transformation. In plant cells with intact cell walls, penetration of the cell wall may be the limiting factor. Microprojectile bombardment currently is the most popular technique for transformation of intact tissues because a considerable amount of information has accumulated regarding the experimental variables and effectiveness of the technique (Sanford, 1990; Birch and Franks, 1991; Batty and Evans, 1992). A range of both monocots and dicots have been transformed through microprojectile bombardment (Batty and Evans, 1992; Weissinger, 1992). Microprojectile bombardment of immature peanut leaflets, with DNA containing *npt*II and *gus* genes under control of the CaMV 35S promoter, has resulted in selection of kanamycin-

resistant, stably transformed callus lines, but no transformed plants (Clemente *et al.*, 1992). It is likely that the few cells in a peanut leaflet competent for plant regeneration are too small a target to expect a reasonable frequency of transformation. Regenerable embryogenic cultures, however, have been successfully transformed via microprojectile bombardment (Ozias-Akins *et al.*, 1992b, 1993). Multiple stably transformed embryogenic cell lines have regenerated more than 100 plants over a period of a few months. The major disadvantage of embryogenic cultures for gene transfer is the 8-month time span from bombardment through selection and plant regeneration. As in soybean (Christou *et al.*, 1989), bombardment of seedling meristems also may result in stable transformation of peanut (Brar *et al.*, 1992). Although bombardment of meristems is a relatively rapid transformation system, the frequency of germ-line transformation events is low and meristems are less amenable to the use of selectable marker genes.

Transformation technology for peanut is developing rapidly. It is likely that a variety of routine protocols encompassing free DNA delivery and *Agrobacterium* transformation of embryogenic and organogenic tissue cultures, explant tissues, and protoplasts soon will be available. It will be important in each case to confirm integration of foreign genes into the genome of the recipient plant by Southern hybridization of the gene sequences to genomic DNA from the putative transgenic plant and to show that the foreign genes are inherited. Genes likely to confer viral resistance (virus coat protein or nucleocapsid genes), insect resistance (*Bacillus thuringiensis* toxin genes), and fungal resistance [peroxidase, thionin, chitinase, glucanase, osmotin-like, and ribosome-inactivating protein genes (Lamb *et al.*, 1992)] are rapidly being tested in other plants and are on the verge of being introduced into peanut. More details regarding gene strategies for specific traits will be covered in subsequent chapters. The next decade will offer an opportunity to test the efficacy of engineered genes for peanut crop protection and should provide an incentive for incorporation of biotechnological approaches in integrated pest management for peanut.

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