Status of the *Arachis* Germplasm Collection in the United States

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ABSTRACT

An extensive working collection of *Arachis* germplasm is maintained by the USDA at the Southern Regional Plant Introduction Sta. in Griffin, GA. Much of this collection is maintained also under long-term seed storage at the Nat. Seed Storage Lab. in Ft. Collins, CO. The working collection consists of 9027 accessions of *A. hypogaea* and 684 accessions of *Arachis* species. About half of the *A. hypogaea* accessions are unimproved landraces collected in the crop's centers of diversity in South America. The other half is comprised of germplasm obtained from countries outside of South America. The U.S. germplasm collection of peanut was the first major germplasm collection to have a working core collection. Research has verified that this core collection can be used to improve the efficiency of germplasm utilization. This has stimulated a great amount of germplasm evaluation work and has resulted in the identification of numerous sources of resistance to several economically significant pathogens. Considerable efforts in the U.S. also have been devoted to the use of wild species of *Arachis* for sources of resistance to pathogens. Programs are ongoing to introgress high levels of resistance or immunity to early (*Cercospora arachidicola* Hori) and late (*Cercosporidium personatum* Berk. & M.A. Curtis) leaf spots, nematodes, and viruses. Genetic resources have been particularly useful in adding disease resistance to peanut cultivars. This has had a significant economic impact on U.S. peanut farmers. The largest impacts have been from the development of cultivars with resistance to *Sclerotinia* blight (*Sclerotinia minor* Jagger), the peanut root-knot nematode [*Meloidogyne arenaria* (Neal) Chitwood race 1], and tomato spotted wilt *Tospovirus*. Use of these resistant cultivars has an estimated economic impact of more that $200 million annually for U.S. peanut producers.

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U.S. Genetic Resources of *Arachis*

The USDA maintains an extensive collection of *Arachis* germplasm. The working collection is maintained by the Plant Genetic Resource Conservation Unit (PGRCU) in Griffin, GA. Much of this collection is maintained also under long-term seed storage condition at the Nat. Seed Storage Lab. in Ft. Collins, CO. The working collection consists of 9027 accessions of *A. hypogaea* L. and 684 accessions of *Arachis* species (R. N. Pittman, pers. commun., 2001). Large *Arachis* species collections in the U.S. are maintained also at Texas A&M Univ. and North Carolina State Univ. (Stalker and Simpson, 1995).

About half of the *A. hypogaea* accessions are unimproved landraces collected from expeditions made to South America, which contains the centers of origin and diversity for peanut. These expeditions were sponsored by the USDA and the Int. Board of Plant Genetic Resources (IBRGR) in cooperation with state experiment stations in the U.S., and by several other countries as described by Isleib *et al.* (1994) and Stalker and Simpson (1995). The collection methods used for peanuts have been published by Hawkes (1976) and Simpson (1984).

Africa is an important secondary center of diversity for *A. hypogaea* (Gibbons *et al.*, 1972). About one-third of the *A. hypogaea* accessions in the U.S. collection originated from Africa. Much of this germplasm was introduced into the U.S. by J. Smartt during the 1960s (Wynne and Gregory, 1981).

In many cases, collected *Arachis* germplasm has been deposited in both the USDA germplasm system and in the Genetic Resource Unit of ICRISAT, Andhra Pradesh, India. 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 of the

ICRISAT collection (Knauft and Ozias-Akins, 1995).

As pointed out by Knauft and Ozias-Akins (1995), additional important germplasm resources in the U.S. exist in the peanut breeding programs of Texas A&M Univ., North Carolina State Univ., the Univ. of Georgia, the Univ. of Florida, USDA, Oklahoma State Univ., Virginia Tech, New Mexico State Univ., and AgraTech Seeds, Inc. Many unique breeding lines developed to have tolerance to various biotic and abiotic stresses are maintained and preserved in these programs.

Germplasm Maintenance, Preservation, and Distribution

Maintenance of *A. hypogaea* accessions is generally straightforward. Seed regeneration is based on the total number of seed available for distribution and the number of requests made by the user community. Both the USDA peanut curator and plant breeders from private industry, universities, and the USDA have cooperated in the regeneration of material to assure adequate seed reproduction. After drying to 5-7% moisture, seed are stored under controlled environmental conditions following the recommendation of Sanders *et al.* (1982) who concluded that the sum of temperature (F) plus relative humidity (RH) should be less than 100 to have optimal seed storage. Peanut seed for the working collection are stored at 4 C and 25% RH. Material which is infrequently requested is stored at -18 C.

Preservation of wild *Arachis* species is much more difficult than for *A. hypogaea*, particularly for accessions that produce few, if any, seed. Approximately 28% of the species accessions produce very few seed, especially the section *Rhizomatosae* species, which are maintained as vegetative materials in the greenhouse (Stalker and Simpson, 1995). Most perennial *Arachis* species can be maintained for many years as original plants or cuttings in greenhouse pots. However, they must be frequently observed and maintained to prevent contamination. An international cooperative effort is underway to insure that these vegetatively propagated species are maintained in multiple environments so that they can be suitably conserved while minimizing the danger of loss (Singh and Simpson, 1994). This effort involves the cooperation of USDA, North Carolina State Univ., Texas A&M Univ., ICRISAT, the Brazilian Corp. for Agric. Res. (EMBRAPA), the Brazilian Nat. Center for Genetic Resources and Biotech. (CENARGEN), the Argentina Nat. Inst. of Agric. Tech. (INTA), and the Argentina Bot. Inst. of the Northeast (IBONE).

At present, approximately 82% of both the cultivated and wild collection is available for distribution (R. N. Pittman, pers. commun., 2001). A backup supply is maintained for about 90% of the *A. hypogaea* collection and about 27% of the wild species collection.

Evaluation Data

Without adequate evaluation data plant breeders cannot know which accessions may be useful parents for cultivar development. Standards for evaluation of *A. hypogaea* accessions have been published by IBPGR and ICRISAT (1992) and the USDA (Pittman, 1995). This involves the characterization of a range of attributes called descriptors. Simpson *et al.* (1992) applied 53 of the IBPGR and ICRISAT descriptors to 2000 accessions collected from 1977 to 1986 in South America and observed a large amount of variation in pod and seed characteristics. Holbrook and Anderson (1993) applied the USDA descriptors to accessions in the U.S. core collection. However, due to the limited resources that have been devoted to germplasm evaluation, little to no evaluation data are available for many accessions. Without these data the potential value of this material will remain unknown.

Development of the Germplasm Resource Information Network (GRIN) <http://www.ars-grin.gov>, a database of descriptor information for each plant introduction in the USDA system, has made it much more efficient to access information regarding the collection. This information can be easily accessed, and plant introductions containing desired characteristics can be ordered for use in research or cultivar development. A pcGRIN version is available also on disk for use when internet access is not available (USDA, 1992).

Development of a Core Collection

Utilization of germplasm collections could be enhanced by the development of more efficient evaluation techniques. Frankel (1984) proposed that a germplasm collection could be reduced to what he termed a core collection. The core collection would be designed to minimize repetitiveness within the collection and should represent the genetic diversity of a crop species. The core collection could serve as a working collection that could be extensively examined, and the accessions excluded from the core collection would be retained as the reserve collection (Frankel, 1984). This proposal was further developed by Frankel and Brown (1984) and Brown (1988, 1989) who described methods to select a core collection using information on the origin and characteristics of the accessions.

The U.S. *A. hypogaea* germplasm collection was the first major germplasm collection to have a working core collection (Holbrook *et al.*, 1993). Data on peanut in the GRIN that were used to select this core collection included country of origin and descriptors for plant type, pod type, seed size, testa color, number of seed per pod, and average seed weight. Available information on accessions varied from only country of origin to information on all seven variables. The U.S. germplasm collection was first stratified by country of origin and then divided into nine sets based on the amount of additional information available for accessions and on the number of accessions per country of origin (Table 1). Seventy percent of this core collection (Sets 4-8) was selected by stratifying by country of origin before using multivariate analysis on morphological data to cluster accessions into groups and then randomly sampling 10% from each group. Because of the lack of morphological data for

some accessions, 29% of this core collection (Sets 2, 3, and 9) was selected using a 10% random sample after stratifying by country of origin. The remaining 1% (Set 1) was a simple random sample.

Accessions included in the core collection are noted in the GRIN. To maximize the usefulness of the peanut core collection, the relationships between the individual accessions as well as the clustering procedure used to develop the core collection are available upon request to the author of this review in two table formats on diskette. The first table lists accessions in numerical order so that the cluster designation for individual accessions can be rapidly determined. The second table lists accessions by clusters so that all accessions within a cluster can be identified.

Table 1. Selection procedures used to select a core collection for the U.S. germplasm collection of *A. hypogaea*.

Set	Total in entire collection	Selection method	Total in core collection
	no.		no.
1	99	Random[a]	10
2	1597	Random by country[b]	160
3	139	Random by country[b]	13
4 & 5	2603	Multivariate clustering[c] - 6 variables	304
6 & 7	756	Multivariate clustering[c] - 5 variables	94
8	1845	Multivariate clustering[c] - 4 variables	210
9	393	Random by country[b]	40
Total	7432		831

[a]10% random sample.

[b]10% random sample after sorting by country of origin.

[c]10% random sample after sorting by country of origin and clustering using the cluster procedure or the fastclus procedure (SAS Inst., 1985).

Evaluation of the Core Collection Theory

The core collection approach to germplasm evaluation is a two-stage approach. The first stage involves examining all accessions in the core collection. This information then is used to decide which clusters of accessions in the entire germplasm collection should be cally, the probability of finding additional accessions with the desired characteristic would be highest in these clusters.

Holbrook and Anderson (1995) used late leaf spot [*Cercosporidium personatum* (Berk. & M.A. Curtis)] resistance data for the entire germplasm collection to retrospectively determine how effective the use of this core collection would have been in identifying sources of resistance in the entire collection. Disease ratings for the core accession(s) representing each cluster were defined as the indicator value for that cluster. Data were examined to determine how many leaf spot-resistant accessions would have been identified by examining the core collection. Data also were examined to determine how many leaf spot-resistant accessions would have been identified by examining all accessions from clusters having a resistant indicator value.

It is important to have data for a number of descriptors available when forming clusters in the core collection (Table 2). The success rate of identifying resistant accessions from groups made by clustering on previous descriptor information is greater than the success rate from groups obtained merely by sampling randomly within countries of origin. The fourth analysis presented in Table 2 shows that the success rate for screening only in resistant clusters in the most fully characterized set is much greater than the success rate for screening all other lines.

Table 2. Comparisons of success rates for finding late leaf spot-resistant accessions in various subsets of the peanut germplasm.

Class	Resistant	Susceptible	Total	Success rate	χ^2
	no.	no.	no.	%	
In core	13	818	831	1.6	0.00ns
Not in core	99	6502	6601	1.5	
Total	112	7320	7432	1.5	
Resist. clusters in Sets 1-9	48	1266	1314	3.7	47.78**
Not in resist. clusters in Sets 1-9	64	6054	6118	1.0	
Total	112	7320	7432	1.5	
Resist. clusters in Sets 4-8	21	143	164	12.8	41.67**
Resist. clusters in Sets 1-3 & 9	27	1123	1150	2.3	
Resist. clusters in Sets 1-9	48	1266	1314	3.7	
Resist. clusters in Sets 4-8	21	143	164	12.8	136.54**
Not in resist. clusters in Sets 4-8	91	7177	7268	1.3	
Total	112	7320	7432	1.5	

[a]Source: Holbrook and Anderson (1995). ns = nonsignificant; ** = significant ($P < 0.01$).

Data in Table 3 also illustrate the improvement in screening efficiency from using this core collection and demonstrates the importance of having descriptor data available to use to cluster accessions before random sampling in the development of a core collection. Screening the entire peanut germplasm collection for resistance to late leaf spot resulted in the identification of one resistant accession for every 66 accessions examined. A similar efficiency would have been observed by screening the core collection. The use of a two-stage core collection screening program greatly improves the efficiency of germplasm screening. Screening of all accessions from all clusters having a resistant indicator value would result in the identification of one resistant accession for every 27 examined. Second-stage testing, considering only those sets developed using the multivariate approach (Sets 4 through 8), would have resulted in the identification of one resistant accession for every eight entries examined.

Holbrook *et al.* (2000b) evaluated the effectiveness of

Table 3. Efficiency of germplasm screening approaches for resistance to late leaf spot in peanut.[a]

Approach	Accessions examined	Resistant accessions identified	Efficiency identified/examined
	no.	no.	no.
Screening the entire collection	7432	112	1/66
Stage I screening core collection	831	13	1/64
Stage II screening clusters w/ resistant indicator(s)[b] from Sets 1-9	1314	48	1/27
Stage II screening clusters w/ resistant indicator(s)[b] from Sets 4-8	164	21	1/8

[a]Source: Holbrook and Anderson (1995).

[b]Disease ratings for the core accession(s) representing each cluster were defined as the indicator value for that cluster.

a two-stage core screening approach in identifying resistance to the peanut root-knot nematode *[Meloidogyne arenaria* (Neal) Chitwood race 1] in the U.S. germplasm collection of *A. hypogaea*. Accessions from 30 clusters having resistant indicator values and from four clusters having susceptible indicator values were tested for resistance in greenhouse trials. The efficiency of identifying resistance to the peanut root-knot nematode in clusters having resistant indicator values was significantly ($P \leq 0.01$) better than the success rate in clusters having susceptible indicator values (Table 4). These results demonstrated that the core collection approach can be used to improve the efficiency of germplasm evaluations.

Utilization of the Peanut Core Collection

The efficiency gained by screening the peanut core collection has greatly increased the use of the peanut germplasm collection. In the U.S., peanut is a regional crop with relatively few individuals involved in breeding and genetic research. Evaluation of core accessions for 24 characteristics (Table 5) has resulted in the identification of numerous sources of resistance to several economically significant pathogens.

Data generated from research with the U.S. core collection have been used to identify the geographical distribution of resistance to five important diseases of peanut (Holbrook and Isleib, 2001). By screening germplasm more intensely from these countries peanut breeders can utilize more efficiently the genes for disease resistance that are available in the U.S. germplasm collection.

Table 4. Comparison of success rates for finding resistance to the peanut root-knot nematode in various subsets of the peanut germplasm collection.[a]

Class	Resistant	Susceptible	Total	Success rate	χ^2
	no.	no.	no.	%	
Clusters with resistant indicator values	227	131	358	63	50.30**
Clusters with susceptible indicator values	31	89	120	26	
Total	258	220	478	54	

[a]Source: Holbrook *et al.* (2000b).

**Significant ($P < 0.01$).

Table 5. Germplasm evaluations for the peanut core collection.

Character	Reference
Cylindrocladium black rot	Isleib *et al.*, 1995
Early leaf spot	Isleib *et al.*, 1995
Fatty acid composition	Hammond *et al.*, 1997
Meloidogyne arenaria	Holbrook *et al.*, 2000a,b
Minimum descriptors	Holbrook, 1997
Percent oil	Holbrook *et al.*, 1998
Preharvest aflatoxin contamination	Holbrook *et al.*, 1997
Rhizoctonia limb rot	Franke *et al.*, 1999
Tomato spotted wilt virus	Anderson *et al.*, 1996

Future Collection Efforts

The peanut core collection has provided a logical subset of the entire germplasm collection that can be examined extensively. Holbrook and Anderson (1993) measured plant descriptor information for all accessions in the core collection. Eight aboveground plant descriptors were evaluated using standard procedures before digging, and nine below-ground descriptors were evaluated similarly after digging. Using these data it was possible to make inferences about the adequacy of the entire collection (Holbrook, 1997). It was concluded that additional *A. hypogaea* accessions should be collected from Columbia, Venezuela, Uruguay, and Bolivia.

Williams (2001) discussed emerging technologies using the geographical information system (GIS) to more effectively study, locate, and conserve *Arachis* genetic resources. He examined existing germplasm collections and the geographical distribution of genetic diversity and concluded that additional collection of wild *Arachis* species is warranted in eastern Bolivia and northwestern Paraguay. Several areas of primary and secondary centers of diversity that warrant further collection of the cultivated species were listed also. Stalker and Simpson (1995) also discussed collection needs, and stated that there is an immediate need for collecting more *A. hypogaea* subsp. *hypogaea* var. *hirsuta* accessions because they are poorly represented in both the USDA and ICRISAT collections. Future collection efforts also were discussed by Singh and Simpson (1994). In addition,

these authors stressed the need to accelerate efforts on characterization and evaluation of extant germplasm so that it can be used effectively and with confidence by breeders.

Since the Convention on Biological Diversity (CBD) in 1993, many countries containing high levels of diversity of *Arachis* have implemented laws regulating access to their genetic resources. Currently, all countries in South America except Paraguay have regulations restricting access to their germplasm. Williams and Williams (2001) discussed innovative, mutually beneficial arrangements which have been developed and used to collect *Arachis* germplasm under CBD regulations. A memorandum of understanding also has been signed by the USDA and ICRISAT to facilitate germplasm exchange between these institutes in light of the CBD regulations (Shands and Bertram, 2000). Both institutions have agreed to forego claims of ownership and intellectual property rights on exchanged germplasm. The same policy applies to germplasm forwarded to state or private institutions when it is passed through the USDA (Williams and Williams, 2001).

Economic Benefits of Genetic Resources

Reducing input costs associated with pest management is becoming increasingly important in the U.S. due to changes in the federal peanut support program (Jordan *et al.*, 1999). Peanut cultivars with disease resistance will allow producers to decrease costs of production and become more competitive with world market prices. Wynne *et al.* (1991) summarized progress in breeding peanut for disease resistance. They concluded that, although several breeding programs had been initiated for developing resistance to diseases during the 1980s, few cultivars had been released by the early 1990s due to the short duration of the programs. However, these efforts had resulted in the identification of many sources of disease resistance in peanut germplasm collections, and they predicted that resistant cultivars would be forthcoming. This prediction is currently being realized. Isleib *et al.* (2001) summarized the use of genetic resources in U.S. peanut cultivar development and concluded that there have been significant economic impacts for the U.S. peanut farmer. The largest impact has been through the development of cultivars with resistance to Sclerotinia blight, root-knot nematodes, and tomato spotted wilt virus. Use of cultivars with these resistances have had an economic impact of more than $200 million annually for U.S. peanut producers.

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