Geographical Distribution of Genetic Diversity in *Arachis hypogaea*

C. C. Holbrook* and T. G. Isleib[1]

ABSTRACT

The U.S. maintains a large (> 8000 accessions) and genetically diverse collection of peanut (*Arachis hypogaea* L.) germplasm. It is costly to screen all accessions within this collection for traits that could be useful in cultivar development. The objective of this research was to identify countries of origin that are rich sources of resistance to important peanut diseases. This would allow peanut breeders to focus their efforts on smaller subsets of the germplasm collection. Accessions in the peanut core collection were evaluated for resistance to late (*Cercosporidium personatum* Berk. & M. A. Curtis) and early (*Cercospora arachidicola* Hori) leaf spot, tomato spotted wilt *Tospovirus* (TSWV), the peanut root-knot nematode [*Meloidogyne arenaria* (Neal) Chitwood race 1], and Cylindrocladium black rot (CBR)[*Cylindrocladium crotalarie* (Loos) Bell & Sobers]. These data then were examined to determine if genes for resistance clustered geographically. Several geographical areas that appear to be rich sources for disease-resistant genes were identified. China had a relatively large number of accessions with resistance to the peanut root-knot nematode. Peru appeared to be a rich source of material with resistance to CBR. Resistance to late leaf spot was more frequent than expected in accessions from Bolivia and Ecuador. Bolivia was also a valuable source of resistance to early leaf spot. Early leaf spot resistance also was more prevalent than expected in accessions from India, Nigeria, and Sudan. India, Israel, and Sudan were valuable origins for material with resistance to TSWV. Accessions with multiple disease resistance were most common in India, Mozambique, and Senegal. This information should enable plant breeders to utilize more efficiently the genes for disease resistance that are available in the U.S. germplasm collection.

Key Words: Cylindrocladium black rot, disease resistance, leaf spot, peanut, root-knot nematode, tomato spotted wilt virus.

The U.S. maintains a collection of over 8000 accessions of *Arachis hypogaea* L. (Pittman, 2000). This collection contains a great amount of genetic diversity; however, it has not been used extensively in cultivar development (Knauft and Gorbet, 1989). Ironically, the size of the collection has limited its use in large part because it is very costly to screen all accessions for traits which could be useful in cultivar development.

A core collection has been developed to represent the U.S. *A. hypogaea* germplasm collection (Holbrook *et al.*, 1993). Theoretically, core collections can be used to more efficiently identify desirable traits, such as disease resistance, in an entire germplasm collection (Frankel, 1984; Brown, 1989). Data on resistance to late leaf spot (*Cercosporidium personatum* Berk. & M.A. Curtis) for the entire *A. hypogaea* collection (Holbrook and Anderson, 1995) and nematode [*Meloidogyne arenaria* (Neal) Chitwood race 1] resistance data from a two-stage core screening approach (Holbrook *et al.*, 2000a,b) were used also to evaluate this core collection. The results of both studies demonstrated that the peanut core collection is a good representative sample and can be used to improve the efficiency of identifying traits in the entire collection.

The objective of this study was to obtain a better understanding of the genetic variability available in the U.S.

[1]Res. Geneticist, USDA-ARS, Coastal Plain Exp. Sta., Tifton, GA 31793 and Prof., Crop Science Dept., North Carolina State Univ., Raleigh, NC 27695, respectively.

*Corresponding author (email: holbrook@tifton.cpes.peachnet.edu).

germplasm collection of peanut. Data for resistance to five diseases were obtained for accessions in the peanut core collection. These data were used then to examine the geographical distribution of resistance.

Materials and Methods

Evaluating for Disease and Pest Resistances

Peanut Root-Knot Nematode. Resistance was evaluated in greenhouse studies in Tifton, GA using the screening technique described by Holbrook *et al.* (1983) with five replications (five plants). Plants were grown in steam-pasteurized loamy sand (85% sand, 11% silt, 4% clay). Each plant was inoculated with 3500 eggs of *M. arenaria* race 1, which had been cultured on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers). Nematode inoculum was prepared using the NaOCl method (Hussey and Barker, 1973) and applied 10 d after planting.

Plants were uprooted and washed clean of soil 90 d after inoculation. The roots were placed in 1000-mL beakers containing 300 mL of phloxine B solution for 3 to 5 min (Daykin and Hussey, 1985). Each plant was indexed for root galls and egg masses on the following scale: 0 = no galls or no egg masses, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, 5 = more than 100 egg masses per root system (Taylor and Sasser, 1978). Accessions were considered resistant if they exhibited a mean egg mass rating less than or equal to 2.5.

Early Leaf Spot. Resistance to *Cercospora arachidicola* Hori was determined in the field at the N. C. Dept. of Agriculture Peanut Belt Research Sta. at Lewiston, NC in the growing season of 1994. The core collection was divided into 16 sets of entries of similar maturities. Sets were planted on 12 and 13 May in 7 × 7 simple lattice designs. The two replicates of a set were grown contiguously. Each plot was a single 3.6-m row with plants spaced 25 cm apart, bordered by two rows of NC 6 at similar spacing. These plots did not receive any fungicide sprays, and natural incidence of early leaf spot was heavy. Plots were rated twice (1 and 15 Sept.) for disease incidence and defoliation using a nine-point scale with 1 indicating no incidence of early leaf spot and 9 indicating 100% incidence or defoliation. Accessions were considered resistant if they exhibited a mean rating less than or equal to 5.0.

Late Leaf Spot. Resistance to *C. personatum* was evaluated in field plots in Tifton, GA. The core collection was divided into five sets of entries with similar maturities. Sets were planted in a randomized complete block design with two replications. Seeds of accessions were planted in two-row plots, 2 m long. Standard cultural practices for peanut production were followed with the exception that no fungicides were used for leaf spot control. Natural incidence of late leaf spot was heavy. Late leaf spot was evaluated on all plots with the 1 to 10 Florida leaf spot disease rating scale (Chiteka *et al.*, 1988; Knauft *et al.*, 1988) in which 1 = no leaf spot and 10 = total plant death due to leaf spot. Accessions were considered resistant if they exhibited a mean rating less than or equal to 6.0.

CBR. Resistance to Cylindrocladium black rot (CBR) [caused by *Cylindrocladium crotalariae* (Loos) Bell & Sobers] was measured in the greenhouse during the winter and early spring of 1994 in Raleigh, NC. The core collection was divided into 16 sets with 49 entries (including checks) in each. Sets were tested in a 7 × 7 lattice design with five replications. The assay was a modification of that used by Black and Beute (1984). Each plot comprised a single plant growing in a 2.54-cm plastic tree-seedling tube filled with inoculated soil. The inoculum was a mixture of microsclerotia from five isolates from North Carolina. An inoculation rate of 25 microsclerotia of *C. parasiticum* per gram of soil was used because it had been determined previously to be sufficient to produce severe stunting in the susceptible cultivar NC 7, while not severely affecting the resistant check NC 3033. Tubes were held in racks with 14 rows of seven tubes each, allowing two sets of 49 tubes to be held in a single rack. Each rack stood in a plastic box with the tips of the tubes immersed in water. Two seeds were planted in each tube to avoid loss of data due to poor germination. The five replicates of the 16 sets were planted at approximately weekly intervals (3, 9, 16, 23, and 30 March).

Ambient temperature in the greenhouse was maintained at 24 to 28 C to promote growth of the fungus. After 8 to 10 wk growth, the seedlings were removed from the tubes. Roots were washed to remove soil and then rated for discoloration and decay on a scale of 0 (no discoloration) to 5 (roots dead and decayed) with half-point increments. Accessions were considered resistant if they exhibited a mean score less than or equal to 1.9.

TSWV. Resistance to tomato spotted wilt virus (TSWV) was evaluated in field plots in Tifton and Attapulgus, GA. The core collection was divided into five sets of entries with similar maturities. Sets were planted in a randomized complete block design with two replications. Seeds of accessions were planted in two-row plots, 2 m long. Standard cultural practices for peanut production were followed, and the natural incidence of TSWV was heavy. Spotted wilt intensity was evaluated in each plot using a disease intensity rating that represents a combination of incidence and severity (Culbreath *et al.*, 1997). The number of 0.3-m portions of row containing severely stunted, chlorotic, wilted or dead plants was counted for each plot and then converted to a percentage of row length. Accessions were considered resistant if they exhibited a mean rating of less than or equal to 40%.

Multiple Disease. Multiple disease resistance (MDR) was estimated by scaling all disease-resistant assessments to a 5-point scale, and then summing the assessments. Accessions in the lowest 15% were considered to have MDR.

Statistical Analysis

The expected number of resistant and susceptible accessions were calculated for each country of origin and for each disease. Expected numbers of resistant accessions were calculated by multiplying the number of accessions in the core collection from each country of origin by the percentage of resistant accessions in the core collection. Chi-square analysis was used to test for significant deviations from expected. The Yates correction term was used since this adds to the accuracy of chi-square analyses when the number of an expected class is small (Strickberger, 1976).

Results and Discussion

The greatest number of entries for resistance to the peanut root-knot nematode originated from China (Table 1). These 11 sources of resistance were greater than what would be expected based on the frequency of accessions from China in the entire collection. We have previously reported that China appears to be valuable geographical

Table 1. Countries contributing the largest number of accessions with resistance to the peanut root-knot nematode and countries contributing more resistant accessions than expected.

Country of origin	Total screened	Resistant accessions Observed	Expected	χ^2
	no.	------- no. -------		
China	31	11	2	41.2**
Japan	13	4	1	6.8**
Zambia	60	4	4	0

**Significantly more (P = 0.01) resistant accessions than expected.

sources of resistance to this nematode (Holbrook *et al.*, 2000a). Second-stage screening of selected groups of accessions from the entire germplasm collection confirmed that observation (Holbrook *et al.*, 2000b). Although peanut is not native to China, it has been cultivated widely there for several centuries (Hammons, 1982), and *M. arenaria* is an important pathogen of peanut in China (Minton and Baugard, 1990).

As previously reported (Holbrook *et al.*, 2000a), Japan also appears to be a valuable geographical source for resistance to the peanut root-knot nematode (Table 1). Second-stage screening of selected groups of germplasm from Japan indicated that this country is an important source for material with resistance to this pathogen (Holbrook *et al.*, 2000b).

More sources of resistance to early leaf spot originated in Bolivia than in any other country. More entries were identified than were expected based on the number of accessions from Bolivia in the entire U.S. collection (Table 2). Bolivia was also a valuable source of resistance to late leaf spot and resistance was more frequent than expected (Table 3). This is not surprising since *A. hypogaea* originated in this area, which is a primary center of diversity; and both leaf spot pathogens also are prevalent in this region. This undoubtedly has resulted in natural and man-assisted selection for resistance to these pathogens.

India, Sudan, and Nigeria also contributed a large number of sources of resistance to early leaf spot, relative to other countries of origin (Table 2). Each of these countries contributed more sources of resistance to early leaf spot than would be expected based on their relative contributions to the U.S. germplasm collection. Peanut germplasm has been grown in these areas with disease pressure from this pathogen for several centuries.

Table 2. Countries contributing the largest number of accessions with resistance to early leaf spot and countries contributing more resistant accessions than expected.

Country of origin	Total screened	Resistant accessions Observed	Expected	χ^2
	no.	-------no.-------		
Bolivia	39	19	8	17.4**
India	46	15	9	4.2*
Zambia	59	15	12	0.6
Sudan	26	10	5	5.1*
Nigeria	21	9	4	6.3*
Israel	41	10	8	0.4

*,**Significantly more (P = 0.05 and 0.01, respectively) resistant accessions than expected.

Table 3. Countries contributing the largest number of accessions with resistance to late leaf spot and countries contributing more resistant accessions than expected.

Country of origin	Total screened	Resistant accessions Observed	Expected	χ^2
	no.	-------no.-------		
Bolivia	39	32	8	86.8**
Argentina	117	14	24	4.8*
Zambia	59	13	12	0.0
Ecuador	9	6	2	6.1**

*,**Significantly (P = 0.05 and 0.01, respectively) different from the expected number of accessions.

Resistance to late leaf spot also was more frequent than expected Ecuador (Table 3). Argentina contributed a large number of sources of resistance to late leaf spot, relative to other countries of origin; however, there were fewer sources of resistance than would be expected based on its relative contribution to the U.S. germplasm collection. Argentina also contributed fewer sources of resistance to early leaf spot and TSWV than expected (data not presented). Peanut production systems in Argentina use higher inputs than are typical of production systems in most other South American countries, and the use of fungicides likely decreased natural selection against the leaf spots. There also have been active peanut research programs in Argentina. Many of the accessions in the U.S. germplasm collection from Argentina were selections from breeding programs. This material may not have been subjected to much, or any, selection pressure for the development of disease resistance.

Argentina, Zambia, and Brazil have contributed the largest number of accessions to the U.S. germplasm collection (Holbrook, 1997). These three countries also contributed a relatively large number of sources of resistance to CBR (Table 4). Peru also contributed a relatively large number of sources of resistance to this pathogen. Peru was the only country of origin where resistance to CBR was more frequent than expected.

Relatively large numbers of accessions with resistance to TSWV originated from India, Israel, Sudan, and Zambia (Table 5). All of these countries are prevalent countries of origin for accessions in the U.S. germplasm collection. However, resistance to TSWV also was more

Table 4. Countries contributing the largest number of accessions with resistance to CBR and countries contributing more resistant accessions than expected.

Country of origin	Total screened	Resistant accessions Observed	Expected	χ^2
	no.	-------no.-------		
Argentina	117	16	21	1.2
Zambia	59	15	11	1.4
Peru	23	11	4	12.8**
Brazil	48	10	9	0.0

**Significantly more (P = 0.01) resistant accessions than expected.

Table 5. Countries contributing the largest number of accessions with resistance to TSWV and countries contributing more resistant accessions than expected.

Country of origin	Total screened	Resistant accessions Observed	Expected	χ^2
	no.	-------no.-------		
India	47	10	4	8.3**
Israel	44	9	4	5.6*
Sudan	27	6	2	6.6**
Zambia	71	6	6	0.0

*,**Significantly more (P = 0.05 and 0.01, respectively) resistant accessions than expected.

frequent than expected in accessions from India, Israel, and Sudan. TSWV does not occur in India; however, peanut has co-evolved in India with peanut bud necrosis virus (PBNV). Although the viruses are distinct and have different thrips vectors, perhaps indirect selection pressure for PBNV also resulted in resistance to TSWV. Occurrence of TSWV in Sudan has not been confirmed. Although TSWV occurs in Israel, it is not an important center of diversity for peanut. Many of the accessions in the U.S. germplasm collection that came from Israel are virginia market-type breeding lines originating in Virginia and/or North Carolina and shared with peanut breeding programs in Israel.

More sources of MDR originated in Zambia and India than in any other country of origin (Table 6). Accessions with MDR were more frequent than expected in accessions from India, Mozambique, and Senegal.

Summary

Countries of origin that are valuable sources of resistance to important diseases of peanut are presented in Table 7. Peanut breeders or pathologists who are interested in sources of resistance to the peanut root-knot nematode should focus their efforts on accessions from China or Japan. Bolivia is an important region for sources of resistance to both leaf spot pathogens. India, Nigeria, and Sudan were also important countries for resistance to early leaf spot, whereas Ecuador was the only other country where resistance to late leaf spot was more prevalent than expected. Peru appears to be the most valuable country for resistance to CBR. Resistance to TSWV was more prevalent than expected in accessions from India, Israel, and Sudan. Researchers who are interested in parents with MDR should consider accessions from India, Mozambique, and Senegal. These observations should enable peanut breeders to more efficiently utilize genetic resources for disease resistance that are available in accessions present in the U.S.germplasm collection.

Table 6. Countries contributing the largest number of accessions with multiple disease resistance and countries contributing more resistant accessions than expected.

Country of origin	Total screened	Resistant accessions Observed	Expected	χ^2
	no.	------- no. -------		
Zambia	59	16	11	2.2
India	46	15	8	6.4*
Mozambique	11	5	2	3.8*
Senegal	8	4	1	7.2**

*,**Significantly more (P = 0.05 and 0.01, respectively) resistant accessions than expected.

Table 7. Valuable origins for disease resistance in the U.S. peanut germplasm collection.

Country of origin	Disease resistance
Bolivia	Early and late leaf spot
China	Peanut root-knot nematode
Ecuador	Late leaf spot
India	Tomato spotted wilt *Tospovirus*, early leaf spot and multiple disease resistances
Israel	Tomato spotted wilt *Tospovirus*
Japan	Peanut root-knot nematode
Mozambique	Multiple disease resistances
Nigeria	Early leaf spot
Peru	Cylindrocladium black rot
Senegal	Multiple disease resistances
Sudan	Tomato spotted wilt *Tospovirus* and early leaf spot

Acknowledgments

The contributions and technical support of Jamie Day, Vickie Hogan, Dannie Mauldin, and Betty Tyler are gratefully acknowledged. This work was supported in part by funds from The Peanut Foundation and the Georgia Peanut Commission.

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