Characterization of ICRISAT Peanut Mini-Core Accessions with Regards to a Molecular Marker Associated with Resistance to Sclerotinia Blight

K.D. Chamberlin[1]*

ABSTRACT

Cultivated peanut, the second most economically important legume crop throughout the United States and the third most important oilseed in the world, is consistently threatened by various diseases and pests. *Sclerotinia minor* Jagger (*S. minor*), the causal agent of Sclerotinia blight, is a major threat to peanut production in the Southwestern U.S., Virginia, and North Carolina and can reduce yield by up to 50% in severely infested fields. Although host plant resistance would provide the most effective solution to managing Sclerotinia blight, limited sources of resistance to the disease are available for use in breeding programs. Peanut germplasm collections are available for exploration and identification of new sources of resistance, but traditionally the process is lengthy, requiring years of field testing before those potential sources can be identified. Molecular markers associated with phenotypic traits can speed up the screening of germplasm accessions. The objective of this study was to characterize the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) mini-core collection with regards to a molecular marker associated with Sclerotinia blight resistance. One hundred twenty-four (124) accessions from the collection were available and genotyped using the SSR marker and 67 were identified as potential new sources of resistance and targeted for further evaluation in field tests for Sclerotinia blight resistance.

Key Words: Peanut, SSR, molecular marker, germplasm, ICRISAT, *Sclerotinia* blight.

Cultivated peanut (*Arachis hypogaea* L.) is a self-pollinated allotetraploid (2n=4x=40) and is the second most economically important legume in the U.S. Peanut is susceptible to many pathogens, with most damage being caused by fungi (Melouk and Backman, 1995). Soil-borne fungi cause diseases that adversely affect peanut health and productivity throughout the growing areas of the United States. Diseases such as pod rot (*Rhizoctonia solani* Kühn, *Pythium myriotylum*), crown rot (*Aspergillus niger* Teigh) and southern blight (*Sclerotium rolfsii* Sacc) occur in all U.S. peanut-producing areas, while others such as Sclerotinia blight (*Sclerotinia minor* Jagger) are limited to certain geographic regions. *Sclerotinia minor* (*S. minor*), the fungus that causes Sclerotinia blight, white mold and watery soft rots of vegetables, or lettuce drop, can infect host plants from 21 families, 66 genera, and 94 species (Porter and Melouk, 1997). Sclerotinia blight was first observed on peanut in Argentina in 1922 and by 1960 reports of *S. minor* causing root and pod rot on peanut were published. Sclerotinia blight is now present in most peanut producing countries of the world but losses are most heavily reported in the U.S. The first reports of *S. minor* in the U.S. were made in Virginia 1971 and then in North Carolina in 1972 (Porter *et al.*, 1982). Sclerotinia blight is of major concern to peanut producers in the Southwest U.S., Virginia and North Carolina. Depending upon severity of field infestation, yield losses due to Sclerotinia blight are typically 10% but may be as high as 50% (Melouk and Backman, 1995). Expensive fungicide applications throughout the growing season are often required for effective disease management. Host plant resistance would provide the most effective solution to managing Sclerotinia blight and limited progress has been made in the development and release of cultivars with enhanced tolerance to the disease (Smith *et al.*, 1991, 1998; Baring *et al*, 2006).

Several factors contribute to the lack of available Sclerotinia blight resistant cultivars. The inheritance mechanism of host resistance is not well understood. Wildman *et al.* (1992) suggested that at least two loci were involved in Sclerotinia blight resistance among genotypes studied. Cytoplasmic factors have also been suggested to be involved in Sclerotinia blight resistance (Coffelt and Porter, 1982). Plant morphology can play an important role in resistance to fungal disease because of the environment required for development and progression (Chappell *et al.*, 1995; Coffelt and Porter, 1982; Coyne *et al.*, 1974; Schwartz *et al.*, 1978). Plant types with a more upright growth habit and open canopy, such as Spanish, appear to be more resistant than those with a dense canopy (such as runner and Virginia types) which allows for temperature reduction and moisture accumulation.

[1]USDA-ARS, 1301 N. Western, Stillwater, OK 74075.
*Corresponding author's E-mail: Kelly.chamberlin@ars.usda.gov

However, the mechanism of resistance among Spanish types is not purely morphological since the Spanish cultivars Pronto and Spanco are as susceptible as many runner types, suggesting contribution by a genetic component.

Another limitation for breeding programs developing *S. minor* resistant cultivars is the lack of known sources of resistance. Cultivated peanut has an extremely narrow genetic base which may have resulted from a single domestication event (Simpson *et al.*, 2001) and subsequent inbreeding among a few select parental lines in commercial breeding programs. The advent of molecular genetics gave peanut breeders new found hope that undiscovered germplasm diversity would soon become apparent and available for use. However, early examinations of the genetic diversity in cultivated peanut revealed little or no polymorphism among the accessions studied (He and Prakash, 1997; Kochert *et al*, 1991; Stalker and Mozingo, 2001). Techniques such as SSR and microsatellite analysis have uncovered some genetic variability in peanut. For example, Hopkins *et al.* (1999) used simple sequence repeat (SSR) primers to uncover six polymorphic SSRs in cultivated peanut and were able to differentiate 15 of 19 accessions tested. Since that discovery, the number of SSR markers has increased (He *et al*, 2005). Chu *et al.* (2007) converted RFLP markers to sequence characterized amplified region (SCAR) markers so as to develop a PCR-based marker system to screen for nematode resistance in peanut. Markers have also been developed for the high-oleate trait (Chu *et al.*, 2009; Chen *et al.*, 2010).

A continued supply of genetic diversity including new or improved variability for target traits is essential for successful crop improvement. Peanut germplasm collections are the best genetic resources for detailed characterization of important traits and are ''gold mines'' for analysis of allelic diversity. The efficiency of peanut improvement programs, whether conventional breeding alone or powered with marker assisted selection (MAS), depends on the accurate phenotypic data which can then be correlated with genotypic marker data. Establishing a highly significant genotype–phenotype association is one of the prerequisites for efficient breeding with MAS. Linkages or associations between target traits genes and molecular markers are detected based on genetic linkage or association mapping experiments. Molecular markers associated with phenotypic traits have proven extremely useful in breeding programs, either for the characterization of members of segregating populations or for the selection of desired parental genotypes so as to pyramid desired traits (Chu *et al.*, 2011). Markers can also be used as tools for screening germplasm collections for possible new sources of desired traits to be incorporated into adapted lines. Chenault *et al.* (2009) used association mapping to identify an SSR marker closely associated with Sclerotinia blight resistance in peanut. Chamberlin *et al.* (2010) used that molecular marker to screen the U.S. peanut mini core collection. In that study, 96 available accessions were genotyped using the marker and 39 total accessions from Spanish, Valencia, runner market types were identified as potential new sources of resistance and targeted for further evaluation in field. The marker was not found to be associated with Sclerotina blight resistance in accessions with a Virginia market-type.

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has over 14,000 accessions in their peanut germplasm collection from which a core collection (Upadhyaya *et al.*, 2003) and a mini-core collection (Upadhyaya *et al.*, 2002) have been developed. Since its creation the ICRISAT peanut mini core has been characterized for drought resistance (Upadhyaya, 2005) and for high oleic acid content (Mukri *et al.*, 2012). The objective of this study was to characterize available accessions from the ICRISAT mini core collection using the molecular marker associated with Sclerotinia blight resistance.

Materials and Methods

Plant Materials

One hundred twenty-four (124) accessions from the ICRISAT peanut mini core collection were available and obtained from R. Pittman (Plant Genetic Resources Conservation Unit, USDA-ARS, Griffin, GA). Seed from all viable accessions (Table 1) were germinated and grown to maturity under greenhouse conditions. Descriptor information (Table 1) was provided by N. Puppala (personal communication).

DNA Extraction and Marker Analysis

DNA was extracted from each genotype listed in Table 1, either from dry, mature seed (Chenault *et al.*, 2007) or from young leaf tissue. In case of the latter, 0.2 g of unfolded leaflet tissue was collected from each plant, de-veined, ground in liquid N_2 to a fine powder and vortexed in 1.5 mL extraction mixture [1:1, extraction buffer (0.1 M Glycine-NaOH, pH 9.0, 50 mM NaCl, 10 mM EDTA, 2% SDS, 1% Na-lauryl sarcosine): phenol-chloroform-isoamyl alcohol (25:24:1)]. Extraction mixtures were shaken vigorously for 10 min and then microfuged for 15 min at 10 K rpm at room

Table 1. List of available accessions of the ICRISAT peanut mini-core collection included in this study along with available descriptor data and the results of molecular marker analysis.

Sample #	ICG #	Country of Origin	Botanical Variety	Growth Habit	Marker Score
1	36	India	*vulgaris*		+
2	76	India	*hypogaea*	runner	−
3	81	Unknown	*vulgaris*		+
4	111	Unknown	*hypogaea*	bunch	−
5	115	India	*fastigiata*		+
6	118	India	*vulgaris*		−
7	163	Unknown	*hypogaea*	runner	−
8	188	India	*hypogaea*	bunch	+
9	513	India	*hypogaea*	bunch	−
10	532	Unknown	*hypogaea*	bunch	−
11	721	USA	*hypogaea*	bunch	−
12	862	India	*hypogaea*	runner	−
13	875	India	*hypogaea*	runner	−
14	928	Unknown	*hypogaea*	runner	−
15	1137	India	*vulgaris*		+
16	1142	Benin	*fastigiata*		+
17	1274	Indonesia	*fastigiata*		+
18	1399	Malawi	*fastigiata*		+
19	1415	Senegal	*vulgaris*		+
20	1519	India	*vulgaris*		+
21	1973	India	*vulgaris*		+
22	2019	India	*vulgaris*		NR
23	2106	India	*vulgaris*		+
24	2511	India	*hypogaea*	runner	−
25	2772	Nigeria	*hypogaea*	bunch	−
26	2773	Tanzania	*hypogaea*	runner	−
27	2777	India	*hypogaea*	runner	−
28	2925	India	*hypogaea*	runner	−
29	3027	India	*hypogaea*	bunch	−
30	3053	India	*hypogaea*	bunch	−
31	3102	India	*vulgaris*		+
32	3240	Uganda	*vulgaris*		+
33	3343	India	*vulgaris*		+
34	3421	India	*vulgaris*		+
35	3584	India	*vulgaris*		+
36	3673	Korea	*fastigiata*		+
37	3746	Argentina	*vulgaris*		+
38	3775	Brazil	*vulgaris*		NR
39	3992	India	*hypogaea*	runner	−
40	4156	Unknown	*hypogaea*	runner	−
41	4343	India	*hypogaea*	runner	−
42	4389	India	*hypogaea*	runner	−
43	4412	USA	*hypogaea*	runner	−
44	4527	Uganda	*hypogaea*	bunch	−
45	4538	India	*hypogaea*	bunch	−
46	4543	Unknown	*vulgaris*		+
47	4598	India	*hypogaea*	bunch	NR
48	4670	Sudan	*fastigiata*		+
49	4684	USA	*vulgaris*		+
50	4729	China	*vulgaris*		+
51	4911	Malawi	*vulgaris*		+
52	4955	India	*vulgaris*		+
53	5195	Sudan	*vulgaris*		+
54	5236	Chile	*vulgaris*		+
55	5286	Zambia	*hypogaea*	bunch	+
56	5475	Kenya	*fastigiata*		+
57	5494	Malaysia	*vulgaris*		+

Table 1. Continued.

Sample #	ICG #	Country of Origin	Botanical Variety	Growth Habit	Marker Score
58	5609	Sri Lanka	*fastigiata*		NR
59	5662	China	*hypogaea*	bunch	−
60	5663	China	*hypogaea*	bunch	−
61	5745	Puerto Rico	*hypogaea*	bunch	−
62	5779	India	*vulgaris*		+
63	5891	India	*hypogaea*	bunch	−
64	6375	Unknown	*vulgaris*		+
65	6402	Unknown	*hypogaea*	bunch	+
66	6407	Zimbabwe	*vulgaris*		+
67	6646	Unknown	*fastigiata*		NR
68	6654	Unknown	*vulgaris*		+
69	6993	Brazil	*hypogaea*	runner	+
70	7153	India	*hypogaea*	runner	−
71	7181	India	*fastigiata*		+
72	8490	Somalia	*hypogaea*	runner	−
73	8760	Zambia	*hypogaea*	runner	−
74	9037	Cote d'Ivoire	*hypogaea*	runner	−
75	9157	Puerto Rico	*vulgaris*		+
76	9249	Mauritius	*vulgaris*		+
77	9315	USA	*fastigiata*		+
78	9418	Martinique	*vulgaris*		+
79	9507	Philippines	*vulgaris*		+
80	9666	India	*hypogaea*	bunch	NR
81	9777	Mozambique	*hypogaea*	bunch	−
82	9809	Mozambique	*vulgaris*		+
83	9842	Tanzania	*hypogaea*	bunch	−
84	9905	Zambia	*hypogaea*	runner	−
85	9961	Unknown	*hypogaea*	bunch	−
86	10092	Zimbabwe	*fastigiata*		+
87	10185	USA	*hypogaea*	bunch	−
88	10384	Nigeria	*vulgaris*		+
89	11249	Tanzania	*vulgaris*		+
90	11322	India	*hypogaea*	bunch	−
91	11426	India	*hypogaea*	runner	+
92	11457	India	*hypogaea*	runner	−
93	11515	China	*vulgaris*		+
94	11651	China	*vulgaris*		+
95	11687	India	*vulgaris*		+
96	11855	Korea	*hypogaea*	bunch	−
97	11862	Korea	*hypogaea*	bunch	−
98	12000	Mali	*hypogaea*	bunch	−
99	12370	India	*hypogaea*	runner	−
100	12682	India	*vulgaris*		+
101	12697	India	*vulgaris*		+
102	12879	Myanmar	*vulgaris*		+
103	12921	Zimbabwe	*vulgaris*		+
104	13099	Unknown	*hypogaea*	runner	−
105	13491	C. African Rep.	*vulgaris*		+
106	13603	Indonesia	*vulgaris*		+
107	13723	Niger	*hypogaea*	runner	−
108	13787	Niger	*hypogaea*	bunch	−
109	13856	Uganda	*fastigiata*		+
110	13858	Uganda	*fastigiata*		+
111	14008	C. African Rep.	*hypogaea*	bunch	−
112	14106	Zaire	*fastigiata*		+
113	14118	Zaire	*vulgaris*		+
114	14127	Zaire	*fastigiata*		+
115	14466	Nigeria	*hypogaea*	bunch	−

Table 1. Continued.

Sample #	ICG #	Country of Origin	Botanical Variety	Growth Habit	Marker Score
116	14475	Nigeria	*hypogaea*	bunch	+
117	14482	Nigeria	*hypogaea*	bunch	NR
118	14523	Unknown	*hypogaea*	bunch	−
119	14630	Brazil	*fastigiata*		+
120	14705	Cameroon	*hypogaea*	bunch	+
121	14710	Cameroon	*fastigiata*		+
122	15190	Costa Rica	*hypogaea*	bunch	−
123	15287	Brazil	*vulgaris*		+
124	15309	Brazil	*fastigiata*		+

temperature. DNA was precipitated from the upper layer of each sample by the addition of 750 µL of isopropanol followed by gentle inversion. DNA was spooled onto a glass hook, washed with 70% ethanol, and allowed to air dry for 15 min at room temperature. Hooks were then placed into tubes containing 1 mL extraction buffer and DNA was re-suspended overnight. DNA suspensions were then incubated with 50 µg Proteinase K for 30 min at 37 C. Proteins and other remaining cellular debris were removed by extraction with phenol-chloroform-isoamyl alcohol (25:24:1) followed by extraction with ½ volume of chloroform to remove remaining phenol. DNA was precipitated by the addition of 750 µL isopropanol, spooled on glass hooks and allowed to air dry for 1 hr at room temperature. DNA was re-suspended in 100 µL of Tris-EDTA buffer and stored at −20 C until further use.

Amplification using primer pPGPseq2E6L (5′ TACAGCATTGCCTTCTGGTG 3′) and primer Marker 3 (5′ GCACACCATGGCTCAGTTATT 3′) was carried out in a PTC-100 thermal cycler (MJ Research, Watertown, MA) under conditions previously reported (Chenault *et al.*, 2009). Bands were identified using Quantity One software (Biorad). Each banding pattern was verified by repeating reactions in triplicate. Resulting bands were scored as previously reported (Chenault *et al.*, 2009). Genotypes possessing the 145 bp band associated with Sclerotinia blight resistance were given a score of (+). Those genotypes carrying only the 100 bp band were given a (−) rating. Those genotypes possessing neither band were not rated (NR).

Results and Discussion

Screening plant germplasm collections often results in new genetic resources for use in breeding programs. The U.S. peanut germplasm collection has been screened for possible sources of resistance to late leaf spot (Holbrook and Anderson, 1995), Meloidogyne arenaria (Holbrook *et al.*, 2000a, 200b), pepper spot (Damicone *et al.*, 2010) and Sclerotinia blight (Chamberlin *et al.*, 2010; Damicone *et al.*, 2009). The ICRISAT peanut collection has also been explored for sources of resistance to leaf spot (Mehan *et al.*, 1994; Subrahamanyam *et al.*, 1995), rust (Subrahamanyam *et al.*, 1995), aflatoxin accumulation (Mehan, 1989), rosette virus (Subrahmanyam *et al.*, 2001), peanut bud necrosis virus (Subrahmanyam *et al.*, 1985) and insect pests (Wightman and Ranga Rao, 1994). Total oil and protein contents have been explored (Upadhyaya *et al.*, 2001) and oleic acid content has been defined (Mukri *et al.*, 2012), as well as crop growth rate, water use efficiency and assimilate partitioning (Nageswara Rao *et al.*, 1994).

In this study, a molecular marker shown to be associated with resistance to Sclerotinia blight in peanut (Chenault *et al.*, 2009) was used to characterize available accessions from the peanut ICRISAT mini core collection. In a previous report, Chenault *et al.* (2009) reported this marker to be significantly associated with resistance seen in 39 peanut genotypes examined in field trials over a 9 year period. Subsequently, this marker has been used to screen the U.S. peanut germplasm mini-core collection and identify possible sources of resistance to Sclerotinia blight (Chamberlin *et al.*, 2010) and was found in accessions of the U.S. core collection shown to be highly resistant in field trials (Damicone *et al.*, 2009, data not shown). Using this marker, successful amplification was achieved for all but 7 available ICRISAT accessions. Lack of amplification for those seven accessions appears to be due to the absence of primer binding site(s) since amplification of the same templates using other primer sets and control primers were successful (data not shown). Data typical of such amplifications is shown in Figure 1 and the genotyping result (marker score) for all accessions is listed in Table 1. Available accessions tested from the ICRISAT collection were from 34 different countries. There was no significant correlation between marker presence and country of origin.

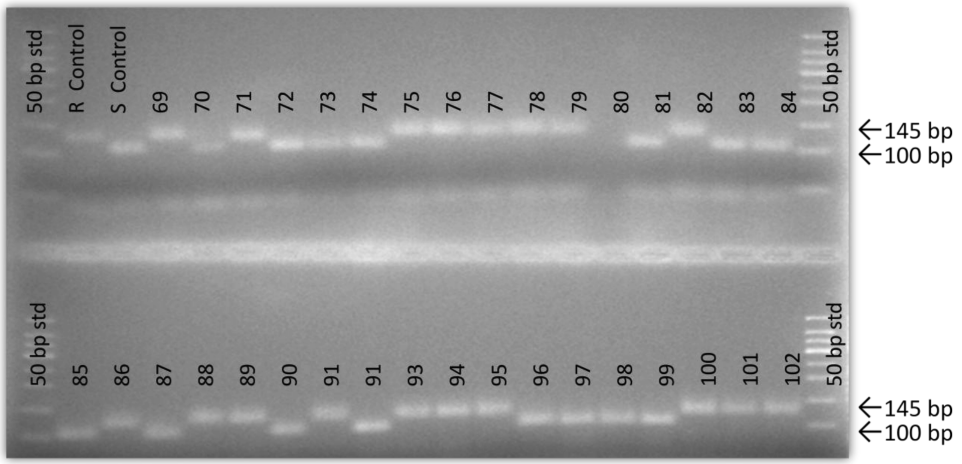

Fig. 1. Example of data from SSR genotyping of ICRISAT peanut mini-core accessions. R control = resistant control cv. Red River Runner; S control = susceptible control cv. Okrun.

Categorized by botanical variety, the ICRISAT peanut collection is comprised of 45% var. *hypogaea*, 35.7% var. *vulgaris*, 16.1% var. *fastigiata*, 0.1% var. *aequitoriana*, 0.14% var. *hirsuta*, and 1.72% var. *peruviana* (Upadhyaya *et al.*, 2001). The composition of the group of ICRISAT peanut mini-core accessions available for this study is 47.6% var. *hypogaea*, 37.1% var. *vulgaris* and 15.3% var. *fastigiata*. Genotyping with the SSR marker associated with Sclerotinia blight resistance in peanut reveals that 67/124 (54%) of the accessions were positive for the presence of that marker. Again, classified by botanical variety, 43/46 (93.5%) of var. *vulgaris* accessions possess the resistance allele, followed by 16/19 (84.2%) of var. *fastigiata* and 7/59 (11.9%) of var. *hypogaea*. The fact that the majority (64%) of the accessions classified as var. *vulgaris* tested positive for the marker is not surprising and is consistent with a previous report of this marker being highly correlated with Sclerotinia blight resistance in Spanish market types (Chenault *et al.*, 2009). It is interesting that, although the marker has also been shown to be tightly linked with resistance in runner market types (var. *hypogaea*), the majority of *hypogaea* accessions are not positive for the marker, and of those that do possess the marker, the majority are listed as having a bunch instead of runner growth habit, which is consistent with Virginia market types. This particular SSR marker has not been shown in the past to be correlated with resistance in Virginia market types (Chenault *et al.*, 2009). Of the var. *hypogaea* accessions that were positive for the marker, three are advanced cultivars, three are landraces, and one is an advanced breeding line. Further investigations with regards to classification of those particular accessions are being conducted to determine if they (1) are true Virginia market types and (2) exhibit elevated levels of resistance to Sclerotinia blight (marker validation). A large majority of accessions classified as var *fastigiata* (market type Valencia) were positive for the marker (84.2%). The marker has been shown to be consistent with resistance found in Valencia market types (Chenault *et al.*, 2009). Previously, the marker used in this study was shown to be significantly associated with resistance to Sclerotinia blight in peanut cultivars and PIs that had been thoroughly evaluated in field trials (Chenault *et al.*, 2009) and the marker was also used to evaluate the U.S. peanut germplasm mini-core (Chamberlin *et al.*, 2010). Furthermore, the presence of this marker has been confirmed in PIs from the U.S. core germplasm collection that were identified as having enhanced resistance to Sclerotinia blight (Damicone *et al.*, 2009) and those new sources of resistance have been incorporated into peanut breeding programs. There are no reports available where ICRISAT accessions have been phenotyped with regards to Sclerotinia blight resistance, but work is currently underway to achieve that task and further validate the use of this marker for resistance screening.

Summary and Conclusions

In this study, 67 ICRISAT mini-core accessions spanning *hypogaea, vulgaris* and *fastigiata* botanical varieties were identified as potential new sources of Sclerotinia blight resistance. Since no reports of field evaluation of the ICRISAT mini-core collection for Sclerotinia blight resistance or susceptibility are available to aid in further validation of this marker as a selection tool for breeding programs, studies are currently underway

toward that end. Furthermore, the success of MAS depends upon the establishment of a significant genotype–phenotype association. Although Chenault *et al.* (2009) established an association of this marker with extensive phenotypic data from cultivar, breeding lines and PIs, more needs to be done to define the extent to which the marker is linked to *S. minor* resistance and to define the corresponding QTL. Currently, recombinant inbred lines (RILs) are being generated specifically for those genetic linkage and association mapping studies. However, the results obtained in this study have identified accessions worthy of such evaluation in the field for *S. minor* resistance and also identified those with low probability of being a source of resistance, reducing the amount of field work required to test the ICRISAT peanut mini-core collection by 46%.

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