Greenhouse Evaluation of Section *Arachis* Wild Species for Sclerotinia Blight and Cylindrocladium Black Rot Resistance

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ABSTRACT

Wild *Arachis* species from section *Arachis* have been promoted as sources of resistance to common peanut diseases and insect pests. The objective of our study was to identify wild *Arachis* species with resistance to Sclerotinia blight and Cylindrocladium black rot (CBR). One hundred and ten accessions/entries from 23 *Arachis* species including *A. hypogaea* were evaluated in the greenhouses at North Carolina State University between January and March of 2010 in a 11×10 rectangular lattice experimental design with 4 replications for Sclerotinia blight and 6 replications for CBR. For the Sclerotinia blight test, seeds were planted in 10 cm clay pots and 8-wk-old plants were inoculated in a mist chamber with BEEM capsules containing the fungus inserted on the petioles of the 4th leaf from the apex on the primary branch. Lesion lengths were measured 4, 5, 6, and 7 d after inoculation, and areas under the disease progress curves (AUDPC) were calculated. For the CBR test, seeds were planted in soil mixed with microsclerotia (25/g) in cone-tainers partly immersed in water. Root damage was recorded after 60 d on a 0–5 proportional scale (0=no decay to 5=completely decayed). Data analysis indicated significant ($p<0.05$) variation among and within *Arachis* species for both diseases. *Arachis glandulifera* exhibited the highest level of Sclerotinia blight resistance followed by *A. correntina, A. herzogii,* and *A. helodes*, although the last three species were not significantly different from *A. hypogaea*. Overall, low genetic variability for Sclerotinia blight resistance was observed among the wild species accessions. For CBR, *A. valida, A. cruziana*, *A. microsperma*, *A. williamsii*, *A. kempff-mercadoi*, *A. kuhlmannii*, *A. helodes*, *A. cardenasii* and *A. correntina* formed the most resistant group with *A. hypogaea* in the most susceptible group. Overall, significant genetic variability for CBR resistance was found among the different wild species accessions. However, not all accessions within a species were resistant to either disease, and most accessions that were resistant to one disease were susceptible to the other.

Key Words: *Arachis hypogaea* L., Groundnut, *Cylindrocladium parasiticum*, *Sclerotinia minor*, Diploid Wild Species, Section *Arachis*.

Among the soilborne diseases of peanut (*Arachis hypogaea* L.), Sclerotinia blight, caused by *Sclerotinia minor* Jagger and Cylindrocladium black rot (CBR), caused by *Cylindrocladium parasiticum* Crous, Wingfield & Alfenas, are important in some peanut production regions of the USA. Individually, each of these diseases can cause annual yield losses of 5–10% (Phipps, 2006), but losses may go up to 50% or more in problem fields (Melouk and Backman, 1995). These two diseases are of great concern to growers as they are difficult or expensive to control. For example, in 2004, about 45% of NC peanut acreage was fumigated for CBR at a cost of approximately $75/ha (Brandenburg *et al.*, 2005). Similarly, chemical sprays to control Sclerotinia blight are very expensive and cost about $100/ha for each application (Hollowell *et al.*, 2008). Moreover, the residue from the chemical sprays is a food safety and environmental issue. In addition to these concerns, seed quality is affected by the two diseases and results in further losses to the growers. Consequently, the most logical and economical option is to provide the growers with high-yielding cultivars which have resistance to both diseases. However, combining resistances has been difficult and cultivars have only been released with resistance to one or the other disease. For example, in the Virginia-Carolina area, a moderately resistant CBR cultivar, NC 12C (Isleib *et al.*, 1997) was found to be susceptible to Sclerotinia blight. Since growers employ resistant cultivars as an important component of managing these two soilborne diseases along with crop rotation and soil fumigation, cultivars with resistance to both these diseases are desired.

Numerous plant introductions within *A. hypogaea* have been identified as sources of disease resistances (Dong *et al.*, 2008; Holbrook and Dong, 2005; USDA, 2007). Use of these sources led to the release of CBR and Sclerotinia blight resistant lines (Branch and Brenneman, 2012; Chamberlin and Melouk, 2009). However, peanut breeders are

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constantly looking for new sources of multiple disease resistances. Wild *Arachis* species from section *Arachis*, which cross hybridize with *A. hypogaea*, have been promoted as sources of resistance to many peanut pests and pathogens (Stalker and Moss, 1987; Subrahmanyam *et al.*, 1985; Stalker and Simpson, 1995; Holbrook, 2001). Developing cultivars with resistance to both CBR and Sclerotinia blight has been a long term goal of the breeding program at N.C. State Univ. The specific objective of this study was to identify new sources of resistance to CBR and Sclerotinia blight among the cross-compatible *Arachis* species. Greenhouse protocols were used to screen a selected list of *Arachis* species in 2010 to meet this objective.

Materials and Methods

Plant Material

Twenty three *Arachis* species including *A. hypogaea*, consisting of a total of 110 genotypes were tested in the greenhouses at N. C. State Univ. in the spring of 2010. The *Arachis* species with the number of accessions of each were listed in Table 1. Seeds from two different sources of the same accession of *A. williamsii* (WiCla 1118), *A. benensis* (Wi 860), and *A. duranensis* (ScBo 15101) were duplicated in this study. The *A. hypogaea* lines used as checks included three cultivars and a Sclerotinia blight resistant germplasm line, N96076L (Isleib *et al.*, 2006). The cultivars included two susceptible checks, Gregory (Isleib *et al.*, 1999), and NC 12C (Isleib *et al.*, 1997); and VA 98R (Mozingo *et al.*, 2000) served as a moderately resistant check. Among the cultivars, VA 98R was also duplicated from a different seed source.

Sclerotinia blight and CBR assays were performed in separate experiments under similar greenhouse conditions (23–27C). All experiments were conducted as incomplete block designs with four replications for Sclerotinia blight and six replications for CBR. The inoculation protocols and data collection were exactly as described in Hollowell *et al.* (2008).

Greenhouse Evaluation of Sclerotinia Blight Reaction

Plants were grown in 10-cm clay pots containing a planting medium of two parts (v:v) steamed commercial topsoil to one part MetroMix 200 (Sun Gro Horticulture, Bellevue, WA) for 8 wk prior to inoculation. The inoculum was prepared by growing *S. minor* isolate P13, which was originally isolated from a diseased peanut plant in Chowan County, NC, on potato dextrose agar (PDA) for 2 d. A plug of PDA colonized by *S. minor* with mycelium side down was placed in a 00 BEEM embedding capsule (Ted Pella, Inc., Redding, CA) with the cap removed and was gently pushed onto a freshly cut petiole on the primary branch of the plant. Inoculated plants were placed on a moisture-retaining mat on the greenhouse bench and were misted daily for 1 min every 2 hr between 6 AM and 8 PM. The bench area was enclosed on the sides and top with plastic sheeting over a PVC frame. After 48 h of incubation, the top cover was removed, but misting continued. Lesion length (mm) was measured 4, 5, 6, and 7 d after inoculation with a digital caliper (Empire Level MFG. Corp., Mukwonago, WI), and area under the disease progress curve (AUDPC) was calculated (Shaner and Finney, 1977).

Table 1. Number of *Arachis* species accessions evaluated and their ploidy and genome designations.

Arachis species	No. of accessions evaluated	Ploidy	Genome
A. batizocoi	5	Diploid	B
A. benensis	4	Diploid	B
A. cardenasii	4	Diploid	A
A. correntina	6	Diploid	A
A. cruziana	1	Diploid	B
A. decora	1	Diploid	A
A. diogoi	2	Diploid	A
A. duranensis	31	Diploid	A
A. glandulifera	2	Diploid	D
A. helodes	6	Diploid	A
A. herzogii	1	Diploid	A
A. hoehnei	2	Diploid	B
A. hypogaea	5	Tetraploid	AB
A. kempff-mercadoi	3	Diploid	A
A. kuhlmannii	15	Diploid	A
A. microsperma	2	Diploid	A
A. monticola	4	Tetraploid	AB
A. palustris	1	Aneuploid	A
A. stenosperma	8	Diploid	A
A. trinitensis	1	Diploid	B
A. valida	1	Diploid	B
A. villosa	3	Diploid	A
A. williamsii	1[a]	Diploid	B

[a]accession WiCla 1118 was evaluated twice.

Greenhouse Evaluation of CBR Reaction

Seeds were planted in plastic cone-tainers, 3.81 cm dia and 20.96 cm in length (Stuewe & Sons, Inc, Corvallis, OR), with a cotton ball placed in the bottom to serve as a wick for water, then filled with a planting medium of two parts (v:v) steamed commercial topsoil, and one part MetroMix 200. The medium was artificially infested with 25 microsclerotia of *C. parasiticum* per g of medium at the time it was mixed. Four isolates of *C. parasiticum* originally obtained from diseased peanut grown in North Carolina were maintained

Table 2. Reaction of *Arachis* species to *Sclerotinia minor* from artificial inoculations in a greenhouse.

Arachis species & accession	Adjusted mean[a] AUDPC	Rank	*Arachis* species & accession	Adjusted mean[a] AUDPC	Rank	*Arachis* species & accession	Adjusted mean[a] AUDPC	Rank	*Arachis* species & accession	Adjusted mean[a] AUDPC	Rank
A. batizocoi	**4.79±0.23$^{\varepsilon}$**	**20**	***A. duranensis***	**4.63±0.10$^{\beta-\varepsilon}$**	**18**	***A. helodes***	**3.95±0.24$^{\alpha\beta\gamma}$**	**4**	***A. microsperma***	**4.51±0.40$^{\alpha-\varepsilon}$**	**14**
A. batizocoi 9484	4.70±0.49^{f-l}		*A. duranensis* 7988	5.42±0.49^{kl}		*A. helodes* 6326	4.01±0.57^{d-k}		*A. microsperma* Grif.15112	4.61±0.57d–l	
A. batizocoi 30080	5.41±0.49^{kl}		*A. duranensis* 15101	4.03±0.49^{d-l}		*A. helodes* 6330	3.32±0.70^{a-f}		*A. microsperma* Grif.15116	4.41±0.57^{d-l}	
A. batizocoi 30081	3.77±0.57^{d-h}		*A. duranensis* 21763	3.61±0.49^{d-g}		*A. helodes* 6331	4.03±0.57^{d-l}		***A. monticola***	**5.08±0.26$^{\varepsilon}$**	**22**
A. batizocoi 30082	5.28±0.49^{h-l}		*A. duranensis* 21764	5.06±0.57^{f-l}		*A. helodes* 6409	4.55±0.57^{d-l}		*A. monticola* 7264	5.44±0.49kl	
A. batizocoi Grif.15031	4.80±0.49f–l		*A. duranensis* 21766	5.47±0.57^{jkl}		*A. helodes* 30029	3.79±0.49^{d-h}		*A. monticola* 30063	4.29±0.57d–l	
A. benensis	**4.79±0.28$^{\gamma\varepsilon}$**	**21**	*A. duranensis* 21767	5.36±0.49^{jkl}		*A. helodes* 30031	3.99±0.57^{d-k}		*A. monticola* PI 263393	5.43±0.49kl	
A. benensis 860	5.29±0.49^{h-l}		*A. duranensis* 30060	4.37±0.57^{d-l}		***A. herzogii***	**3.83±0.57$^{\alpha\beta}$**	**3**	*A. monticola* PI 405933	5.17±0.49g–l	
A. benensis 35005	3.67±0.49^{d-h}		*A. duranensis* 30064	5.31±0.49^{i-l}		*A. herzogii* 36029	3.83±0.57^{d-i}		***A. palustris***	**5.26±0.49$^{\delta\varepsilon}$**	**23**
A. benensis 35006	5.30±0.49^{h-l}		*A. duranensis* 30065	4.62±0.49^{e-l}		***A. hoehnei***	**4.41±0.38$^{\beta-\varepsilon}$**	**13**	*A. palustris* 13023	5.26±0.49^{g-l}	
A. benensis 860	4.92±0.70^{e-l}		*A. duranensis* 30067	2.89±0.70^{a-d}		*A. hoehnei* 9094	3.32±0.57^{b-f}		***A. stenosperma***	**4.32±0.19$^{\alpha-\varepsilon}$**	**9**
A. cardenasii	**4.61±0.27$^{\beta-\varepsilon}$**	**16**	*A. duranensis* 30068	4.33±0.49d–l		*A. hoehnei* 30006	5.50±0.49^{l}		*A. stenosperma* 408	3.94±0.49d–j	
A. cardenasii 10017	4.00±0.49^{d-k}		*A. duranensis* 30069	5.26±0.49^{g-l}		***A. hypogaea***	**3.98±0.23$^{\alpha\beta\gamma}$**	**5**	*A. stenosperma* 409	4.30±0.49d–l	
A. cardenasii 36018	4.45±0.49^{d-l}		*A. duranensis* 30070	4.67±0.57^{e-l}		*A. hypogaea* Gregory	4.74±0.49f–l		*A. stenosperma 7377*	4.16±0.70^{d-l}	
A. cardenasii 36020	4.81±0.57^{f-l}		*A. duranensis* 30071	5.38±0.99^{e-l}		*A. hypogaea* N96076L	2.10±0.49ab		*A. stenosperma* 7382	3.96±0.49d–j	
A. cardenasii 36032	5.19±0.57^{g-l}		*A. duranensis* 30072	4.03±0.70^{d-l}		*A. hypogaea* VA 98R	4.76±0.57e–l		*A. stenosperma 7377*	4.88±0.49^{f-l}	
A. correntina	**3.49±0.22$^{\alpha\beta}$**	***2***	*A. duranensis* 30074	5.18±0.49^{g-l}		*A. hypogaea* NC 12C	4.08±0.57d–l		*A. stenosperma 9017*	4.17±0.57^{d-l}	
A. correntina 7830	2.17±0.49^{abc}		*A. duranensis* 30075	4.75±0.70^{d-l}		*A. hypogaea* VA 98R	4.21±0.49^{d-l}		*A. stenosperma* 13824	5.47±0.57^{jkl}	
A. correntina 7897	4.60±0.57^{d-l}		*A. duranensis* 30077	5.07±0.57^{f-l}		***A. kempff-mercadoi***	**4.36±0.33$^{\alpha-\delta}$**	**10**	*A. stenosperma* 13693	3.70±0.49^{d-h}	
A. correntina 9530	4.08±0.49^{d-l}		*A. duranensis* 30078	4.86±0.49^{f-l}		*A. kempff-mercadoi* 30084	4.75±0.57e–l		***A. trinitensis***	**4.54±0.57$^{\alpha-\varepsilon}$**	**15**
A. correntina 19616	3.82±0.57^{d-h}		*A. duranensis* 30079	4.45±0.57^{d-l}		*A. kempff-mercadoi* 30088	4.04±0.57d–l		*A. trinitensis* 1117	4.54±0.57^{d-l}	
A. correntina 36000	1.83±0.49^{a}		*A. duranensis* 36002	4.83±0.49^{f-l}		*A. kempff-mercadoi* 35001	4.28±0.57^{d-l}		***A. valida***	**4.74±0.49$^{\alpha-\varepsilon}$**	**19**
A. correntina 36001	4.44±0.57^{d-l}		*A. duranensis* 36003	5.44±0.49^{kl}		***A. kuhlmannii***	**4.38±0.14$^{\beta-\varepsilon}$**	**11**	*A. valida* 30147	4.74±0.49^{f-l}	
A. cruziana	**4.61±0.70$^{\alpha-\varepsilon}$**	**17**	*A. duranensis* 36005	4.34±0.70^{d-l}		*A. kuhlmannii* 6404	4.33±0.49d–l		***A. villosa***	**4.17±0.34$^{\alpha\beta\gamma}$**	**7**
A. cruziana 36024	4.61±0.70^{d-l}		*A. duranensis* 36006	3.91±0.57^{d-j}		*A. kuhlmannii* 6408	4.42±0.49d–l		*A. villosa* Grif.7726	3.58±0.70^{b-g}	
A. decora	**4.28±0.57$^{\alpha\beta\gamma}$**	**8**	*A. duranensis* 36036	3.47±0.57^{b-f}		*A. kuhlmannii* 7639	3.87±0.49d–i		*A. villosa* Grif.7727	4.93±0.49^{f-l}	
A. decora 9953	4.28±0.57^{d-l}		*A. duranensis* 10038 ll.	4.21±0.57^{d-l}		*A. kuhlmannii* 8888	4.57±0.49e–l		*A. villosa* 22585	4.00±0.57^{d-k}	
A. diogoi	**4.06±0.40$^{\alpha\beta}$**	**6**	*A. duranensis* Grif.15035	4.05±0.70^{d-l}		*A. kuhlmannii* 8889	5.12±0.49g–l		***A. williamsii***	**4.39±0.38$^{\alpha-\delta}$**	**12**
A. diogoi 10602	3.90±0.57^{d-j}		*A. duranensis* Grif.15036	4.67±0.57^{e-l}		*A. kuhlmannii 8916*	4.83±0.49f–l		*A. williamsii* 1118	4.32±0.49^{d-l}	
A. diogoi 30001	4.23±0.57^{d-l}		*A. duranensis* Grif.15037	5.15±0.57^{g-l}		*A. kuhlmannii* 8935	3.22±0.57a–e		*A. williamsii* 1118	4.46±0.57^{d-l}	
			A. duranensis Grif.15038	4.63±0.49^{e-l}		*A. kuhlmannii* 9214	3.62±0.70b–h				
			A. duranensis 15101	4.17±0.49^{f-l}		*A. kuhlmannii* 9230	5.25±0.57g–l				

Table 2. Continued.

Arachis species & accession	Adjusted mean[a] AUDPC	Rank	*Arachis* species & accession	Adjusted mean[a] AUDPC	Rank	*Arachis* species & accession	Adjusted mean[a] AUDPC	Rank	*Arachis* species & accession	Adjusted mean[a] AUDPC	Rank
			A. glandulifera	**3.22±0.45**[z]	**1**	*A. kuhlmannii* 9470	4.43±0.49	d–l			
			A. glandulifera 30099	3.31±0.70	a–f	*A. kuhlmannii* 30008	3.24±0.49	b–e			
			A. glandulifera 30100	3.13±0.57	a–d	*A. kuhlmannii* 30017	5.32±0.57	g–l			
						A. kuhlmannii 30034	4.88±0.49	f–l			
						A. kuhlmannii 30035	4.17±0.57	d–l			
						A. kuhlmannii Grif.7693	4.45±0.49	d–l			

[a]Accession means followed by the same letter are not different (P<0.05) by t-test.

in culture on PDA. Inoculum was prepared by transferring cultures to PDA and incubating at room temperature for 6 wk in the dark. Microsclerotia from cultures of each of the four isolates were recovered using a blender and water, sieved separately, later mixed in a water suspension and quantified (Black and Beute, 1984; Phipps *et al.*, 1976).

Cone-tainers in racks were placed in plastic trays, and plants were grown for 8 wk in the greenhouse. Water in each tray was maintained at a height of approximately 10 cm (approx. 3 cm from the bottom of the tubes) to provide adequate soil moisture. The root system of any plant that died before data collection was washed and plated on PDA to determine whether *C. parasiticum* was present in the decaying roots. At 60d, surviving plants were removed from the cone-tainers, and the roots were washed and rated for degree of decay on a 0–5 proportional scale (0=no lesions or decay, 1=few lesions on secondary roots and / or a few small lesions on taproot, 3=many lesions on secondary roots and many lesions on the taproot and with several secondary roots missing, 5=completely decayed roots with most secondary roots and part of taproot missing, with 2 and 4=intermediate levels of severity, respectively (Black and Beute, 1984; Rowe and Beute, 1975). Any plant that died prior to the end of the experiment and was confirmed to have been infected with *C. parasiticum* received a score of 5. Any plant that died early, but did not harbor the fungus was considered a missing value.

Data Analysis

Lesion lengths, AUDPC values, and root rot scores from the greenhouse experiments were subjected to analysis of variance for the incomplete block design using the general linear models procedure (PROC GLM) of SAS v8.0 statistical software (SAS Institute, Cary, NC). If the effects of blocks within reps were not significant at $P<0.05$, then blocks were dropped from the model and the data were analyzed as if they came from a randomized complete block experiment. The AUDPC values were log transformed to stabilize the error variance among the means and adjusted means were computed using the final model. For the duplicated species accessions, contrasts of means were constructed to determine if the duplicated sample means are different from each other.

Results and Discussion

Reaction of *Arachis* Species to Sclerotinia Blight

Petioles of plants inoculated with *S. minor* in the greenhouse exhibited water-soaked symptoms after

24 hr, and lesions were found on stems by 3 to 4 d after inoculation. In some cases, the petiole of an inoculated plant became water soaked, but no lesion developed. Occasionally, petioles and capsules fell off due to the weakening of petioles after attack by the fungus. Usually the fungus had already invaded the stem, and lesions developed as observed on other plants. Otherwise, data for the plant was recorded as missing (Hollowell *et al.*, 2008).

Significant variation among the 23 *Arachis* species and within accessions of species were observed ($P<0.05$) for lesion length (Table 2). Replications within blocks did not have significant effects ($P<0.05$), and as such, blocks were dropped from the analysis. As a group, *A. glandulifera* exhibited the lowest mean AUDPC of 3.22 ± 0.45 and ranked as the most resistant among the 23 species. The two accessions of this species, KGSSc 30099 and KGSSc 30100, had mean AUDPC values significantly lower than the checks (Table 2). *Arachis glandulifera* is the sole D genome species in the genus (Stalker, 1991), but it is cross compatible with *A. hypogaea* (AABB genomes) and thus offers the possibility that resistance may be transferred. Additionally, *A. correntina* (A genome) with a mean AUDPC value of 3.49 ± 0.22 (mean of six accessions) had the highest levels of resistance as a species and significantly lower mean value than the cultivars. Two different accessions of this species, KSBVn 36000 and KRiP 7830 had the lowest mean AUDPC values among the diploid species accessions (Table 2) and were the most resistant of all the diploid species accessions evaluated in this study. Although as a group *A. batizocoi* was susceptible, one of its accessions, KGBSPSc 30081, had mean AUDPC of 3.77 ± 0.57 which was significantly lower than the cultivars as a group. Additionally, *A. herzogii* and *A. helodes* ranked higher than *A. hypogaea* based on the mean AUDPC values, although they are not significantly different from the latter (Table 2). Among the *A. hypogaea* lines, the partially resistant Sclerotinia blight germplasm line, N96076L (Isleib *et al.*, 2006) exhibited the lowest mean AUDPC value (2.10 ± 0.49) as expected. In this study, *A. duranensis* (A genome) contained the highest number of accessions (31) with a large amount of variation for Sclerotinia blight infection. One of the accessions, KGBSPSc 30067, exhibited the least mean AUDPC value of 2.89 ± 0.70 within this group. However, many of the other accessions of this species were not significantly different from *A. hypogaea*. For those species with duplicated samples of the same accession, for example *A. williamsii* accession WiCla 1118 (Tables 2), the mean AUDPC values were not significantly different. Overall, limited useful genetic variability for Sclerotinia blight resistance was observed among the wild species accessions, but some individual accessions of *A. correntina* (KSBVn 36000 and KRiP 7830) have promising levels of Sclerotinia blight resistance.

Reaction of *Arachis* Species to CBR

Significant variation ($P<0.05$) among and within species accessions was observed for CBR root decay (Table 3). The mean root rot scores suggested that *A. valida*, *A. microsperma*, and *A. cruziana* as the most resistant species. The next best group of species included, *A. williamsii*, *A. helodes* and *A. kempff-mercadoi*. *Arachis williamsii* accession, WiCla 1118, which was duplicated, had similar levels of root rot that did not differ significantly. The root rot scores of *A. hypogaea* checks were higher than most entries tested in this study. It is also interesting to note that N96076L which was resistant to Sclerotinia blight but susceptible to CBR. Individual accessions of several other species such as, *A. batizocoi* (K 9484, KGBSPSc 30081 and KGBSPSc 30082), *A. kuhlmannii* (Grif.7693, VSGr 6404), and *A. stenosperma* (VSMGeSv 7377), and *A. correntina* (KSBVn 36000) also exhibited low levels of mean root rot (Table 3). Additionally, several *A. duranensis* accessions also had low mean root rot including the A genome donor accession, KGBSPSc 30067.

Greenhouse evaluations are less expensive and less laborious compared to field evaluations and may be more reliable for preliminary screening of germplasm for disease resistances. Additionally, good performance in the greenhouse assay can be used to supplement field results in the selection of lines for disease resistance. Hollowell *et al.* (2008) reported, in a comparison of multiple-year greenhouse assays with field data, a correlation of $r=0.83$ ($P<0.0001$) for CBR, suggesting that the greenhouse assay is a good predictor of field performance. However, the assay for Sclerotinia blight was less reliable as a predictor of field performance as the correlation was only 0.35 ($P<0.0001$). Similar greenhouse screening methods were used in the early identification of CBR resistant germplasm in peanut (Pataky *et al.*, 1983).

Developing genetic resistance to these diseases will offer growers the economic stability to manage the crop with less input costs. New cultivars with partial resistance to CBR and Sclerotinia blight are being made available to growers (Branch and Brenneman, 2012; Chamberlin and Melouk, 2009). However, new sources of resistances are needed to develop more cultivars with better levels of resistance to these two diseases. As shown in this study, several diploid wild species have exhibited resistance to Sclerotinia blight and CBR in the

Table 3. Reaction of *Arachis* species to Cylindrocladium black rot (CBR) from artificial inoculations in a greenhouse.

Arachis species & accession	Adjusted mean[a] root rot score	Rank	*Arachis* species & accession	Adjusted mean[a] root rot score	Rank
A. batizocoi	**1.50±0.20$^{\alpha\beta}$**	**12**	***A. duranensis***	**1.52±0.09$^{\alpha\beta}$**	**13**
A. batizocoi 9484	0.94±0.48^{ab}		*A. duranensis* 7988	1.33±0.39^{abc}	
A. batizocoi 30080	3.22±0.48$^{h–m}$		*A. duranensis* 15101	0.62±0.43^{a}	
A. batizocoi 30081	1.36±0.43$^{a–d}$		*A. duranensis* 21763	3.23±0.43$^{j–m}$	
A. batizocoi 30082	1.00±0.39^{ab}		*A. duranensis* 21764	1.41±0.43$^{a–e}$	
A. batizocoi Grif.15031	0.99±0.43^{ab}		*A. duranensis* 21766	3.72±0.48^{lm}	
A. benensis	**1.91±0.25$^{\alpha\beta}$**	**17**	*A. duranensis* 21767	1.33±0.39$^{a–d}$	
A. benensis 860	0.89±0.68^{ab}		*A. duranensis* 30060	1.19±0.43^{ab}	
A. benensis 35005	2.79±0.43$^{f–m}$		*A. duranensis* 30064	0.97±0.48^{ab}	
A. benensis 35006	2.76±0.43$^{e–m}$		*A. duranensis* 30065	1.00±0.39^{ab}	
A. benensis 860	1.19±0.43^{b}		*A. duranensis* 30067	0.94±0.48^{ab}	
A. cardenasii	**1.27±0.17$^{\alpha}$**	**8**	*A. duranensis* 30068	2.94±0.48$^{h–m}$	
A. cardenasii 10017	1.17±0.39^{ab}		*A. duranensis* 30069	0.93±0.68^{ab}	
A. cardenasii 36018	1.67±0.39$^{a–f}$		*A. duranensis* 30070	1.01±0.43^{ab}	
A. cardenasii 36020	1.17±0.39^{ab}		*A. duranensis* 30071	0.97±0.48^{ab}	
A. cardenasii 36032	1.00±0.39^{ab}		*A. duranensis* 30072	1.02±0.43^{ab}	
A. correntina	**1.30±0.18$^{\alpha}$**	***9***	*A. duranensis* 30074	2.79±0.43$^{f–m}$	
A. correntina 7830	1.67±0.39$^{a–f}$		*A. duranensis* 30075	1.03±0.43^{ab}	
A. correntina 7897	1.19±0.48^{ab}		*A. duranensis* 30077	1.21±0.43^{ab}	
A. correntina 9530	1.17±0.39^{ab}		*A. duranensis* 30078	2.49±0.48$^{b–l}$	
A. correntina 19616	1.39±0.55$^{a–e}$		*A. duranensis* 30079	1.03±0.43^{ab}	
A. correntina 36000	1.24±0.48^{abc}		*A. duranensis* 36002	0.76±0.48^{a}	
A. correntina 36001	1.17±0.39^{ab}		*A. duranensis* 36003	1.81±0.43$^{a–g}$	
A. cruziana	**1.00±0.39$^{\alpha}$**	**3**	*A. duranensis* 36005	1.17±0.39^{ab}	
A. cruziana 36024	1.00±0.39^{ab}		*A. duranensis* 36006	1.79±0.43$^{a–g}$	
A. decora	**2.28±0.55$^{\alpha\beta\gamma}$**	**18**	*A. duranensis* 36036	2.02±0.43$^{a–h}$	
A. decora 9953	2.28±0.55$^{b–k}$		*A. duranensis* 10038 ll.	1.67±0.39$^{a–f}$	
A. diogoi	**1.68±0.31$^{\alpha\beta}$**	**15**	*A. duranensis* Grif.15035	3.52±0.48^{klm}	
A. diogoi 10602	2.17±0.39$^{b–j}$		*A. duranensis* Grif.15036	0.48±0.68^{a}	
A. diogoi 30001	1.19±0.48^{ab}		*A. duranensis* Grif.15037	1.06±0.48^{ab}	
			A. duranensis Grif.15038	2.00±0.39$^{a–g}$	
			A. duranensis 15101	0.99±0.43^{ab}	
			A. glandulifera	**2.78±0.32$^{\gamma}$**	**22**
			A. glandulifera 30099	2.72±0.48$^{e–m}$	
			A. glandulifera 30100	2.83±0.43$^{g–m}$	

[a]Accession means followed by the same letter are not different ($P<0.05$) by t-test.

greenhouse tests and offer new germplasm to breed for resistance to these two diseases. Overall, in this study, the *A. batizocoi* accession KGBSPSc 30081 and *A. correntina* accession KSBVn 36000, appeared to contain resistance to both Sclerotinia blight and CBR and are promising sources to breed for resistance to both these diseases. Some of the CBR resistant species such as *A. valida*, *A. williamsii* and *A. cruziana* are B genome species, and it is likely that amphidiploids can be produced with other CBR resistant A genome species such as *A. duranensis* accession KGBSPSc 30067. Similarly, the CBR resistant B genome species accessions can be hybridized with Sclerotinia blight resistant A genome species accessions to produce amphidiploids with resistance to both these diseases. Also, the *A. duranensis* accession KGBSPSc 30067, which exhibited resistance to both diseases in this study, has been reported as the A genome donor (Kochert *et al.*, 1996) to *A. hypogaea* and offers the possibility that some of the PIs within *A. hypogaea* may contain higher levels of resistance to both CBR and Sclerotinia blight than has been reported. This observation prompted speculation that an evaluation of *A. hypogaea* PIs collected in close proximity to the location where KGBSPSc 30067 was found may provide resistant germplasm. In the present study, the partially resistant Sclerotinia blight germpalsm line, N96076L, as expected, exhibited resistance where as the diploid

Table 3. Extended.

Arachis species & accession	Adjusted mean[a] root rot score	Rank	*Arachis* species & accession	Adjusted mean[a] root rot score	Rank
A. helodes	**1.18±0.17$^{\alpha}$**	**7**	***A. microsperma***	**1.00±0.30$^{\alpha}$**	**2**
A. helodes 6326	1.16±0.43^{ab}		*A. microsperma* Grif.15112	1.03±0.43^{ab}	
A. helodes 6330	1.17±0.39^{ab}		*A. microsperma* Grif.15116	0.96±0.43^{ab}	
A. helodes 6331	0.97±0.48^{ab}		***A. monticola***	**1.46±0.20$^{\alpha\beta}$**	**10**
A. helodes 6409	1.62±0.43$^{a–f}$		*A. monticola* 7264	1.67±0.39$^{a–f}$	
A. helodes 30029	1.17±0.39^{ab}		*A. monticola* 30063	1.16±0.43^{ab}	
A. helodes 30031	1.00±0.39^{ab}		*A. monticola* PI 263393	1.50±0.39$^{a–e}$	
A. herzogii	**1.50±0.39$^{\alpha\beta}$**	**11**	*A. monticola* PI 405933	1.50±0.39$^{a–e}$	
A. herzogii 36029	1.50±0.39$^{a–e}$		***A. palustris***	**2.39±0.43$^{\beta\gamma}$**	**20**
A. hoehnei	**3.00±0.28$^{\gamma}$**	**23**	*A. palustris* 13023	2.39±0.43$^{b–l}$	
A. hoehnei 9094	3.83±0.39^{m}		***A. stenosperma***	**1.77±0.16$^{\alpha\beta}$**	**14**
A. hoehnei 30006	2.17±0.39$^{b–j}$		*A. stenosperma* 408	2.19±0.43$^{b–j}$	
A. hypogaea	**2.75±0.19$^{\gamma}$**	**21**	*A. stenosperma* 409	2.65±0.55$^{c–m}$	
A. hypogaea Gregory	2.67±0.39$^{e–m}$		*A. stenosperma* 7377	1.01±0.48^{ab}	
A. hypogaea N96076L	3.22±0.48$^{h–m}$		*A. stenosperma* 7382	2.50±0.39$^{e–l}$	
A. hypogaea VA 98R	3.17±0.39$^{i–m}$		*A. stenosperma* 7762	1.50±0.39$^{a–e}$	
A. hypogaea NC 12C	2.67±0.39$^{e–m}$		*A. stenosperma* 9017	1.83±0.39$^{a–g}$	
A. hypogaea VA 98R	3.03±0.43$^{a–i}$		*A. stenosperma* 13824	0.90±0.55^{ab}	
A. kempff-mercadoi	**1.11±0.23$^{\alpha}$**	**5**	*A. stenosperma* 13693	1.59±0.43$^{a–e}$	
A. kempff-mercadoi 30084	1.17±0.39^{ab}		***A. trinitensis***	**2.35±0.55$^{\alpha\beta\gamma}$**	**19**
A. kempff-mercadoi 30088	1.00±0.39^{ab}		*A. trinitensis* 1117	2.35±0.55$^{b–l}$	
A. kempff-mercadoi 35001	1.17±0.39^{ab}		***A. valida***	**0.99±0.48$^{\alpha}$**	**1**
A. kuhlmannii	**1.22±0.11$^{\alpha}$**	**6**	*A. valida* 30147	0.99±0.48^{ab}	
A. kuhlmannii 6404	1.00±0.39^{ab}		***A. villosa***	**1.26±0.20$^{\alpha}$**	**16**
A. kuhlmannii 6408	1.17±0.39^{ab}		*A. villosa* Grif.7726	1.05±0.55^{ab}	
A. kuhlmannii 7639	1.43±0.43$^{a–e}$		*A. villosa* Grif.7727	1.50±0.39$^{a–e}$	
A. kuhlmannii 8888	1.67±0.39$^{a–f}$		*A. villosa* 22585	1.22±0.43^{ab}	
A. kuhlmannii 8889	1.23±0.43^{abc}		***A. williamsii***	**1.09±0.32$^{\alpha}$**	**4**
A. kuhlmannii 8916	1.36±0.43$^{a–e}$		*A. williamsii* 1118	1.22±0.48^{abc}	
A. kuhlmannii 8935	1.17±0.39^{ab}		*A. williamsii* 1118	0.96±0.43^{ab}	
A. kuhlmannii 9214	1.17±0.39^{ab}				
A. kuhlmannii 9230	1.33±0.39^{abc}				
A. kuhlmannii 9470	0.55±0.48^{a}				
A. kuhlmannii 30008	1.83±0.39$^{a–g}$				
A. kuhlmannii 30017	1.17±0.39^{ab}				
A. kuhlmannii 30034	1.00±0.39^{ab}				
A. kuhlmannii 30035	1.16±0.43^{ab}				
A. kuhlmannii Grif.7693	1.00±0.39^{ab}				

[a]Accession means followed by the same letter are not different ($P<0.05$) by t-test.

wild species *A. cardenasii* (GKP 10017) which was involved in the parentage of N96076L was susceptible to Sclerotinia blight. N96076L has several ancestors, and not all of them may have contributed the resistance. Further, discrepancies in the reaction of the same *Arachis* species or the same accession within a species under different environmental conditions were reported by Subrahmanyam *et al.* (1985). They indicated that the differences in disease reactions may be due to the interaction of the host, pathogen and the environment and also due to the variation within the pathogen. However, the recent multiple disease resistant cultivar releases, Bailey (Isleib *et al.*, 2011), and Tifguard (Holbrook *et al.*, 2008) trace their resistances to diploid *Arachis* species indicating the potential benefits of wild species research and warrants their use for peanut improvement.

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