Screening of the Argentinean INTA peanut core collection with a molecular marker associated with resistance to *Sclerotinia minor* Jaggar

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ABSTRACT

Cultivated peanut, 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 many countries and can reduce yield by up to 50% in severely infested fields. Host plant resistance will provide the most effective solution to managing Sclerotinia blight, but 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 genotype the peanut core collection of the Instituto Nacional de Tecnología Agropecuaria (INTA) Manfredi, Argentina, with a molecular marker associated with Sclerotinia blight resistance. One hundred and fifty-four (154) accessions from the collection were available and genotyped using the Simple Sequence Repeat (SSR) marker. Accessions from each botanical variety type represented in the core collection were identified as new potential sources of resistance and targeted for further evaluation in field tests for Sclerotinia blight resistance.

Key Words: Peanut, Sclerotinia blight, Resistance, Argentina

Cultivated peanut (*Arachis hypogaea* L.) is one of the most economically important legumes in the world. 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, often requiring management by fungicide treatment throughout the growing season which is expensive to the producer and harmful to the environment. Sclerotinia blight [causal agent *Sclerotinia minor* (Jagger)] is of major concern to peanut producers in many parts of the world, including Argentina. Depending upon the severity of field infestation, yield losses due to Sclerotinia blight may be as high as 50% (Melouk and Backman,1995).

Host plant resistance provides the most effective solution to managing Sclerotinia blight, but 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; Baring *et al*., 2013; Melouk *et al*., 2013; Chamberlin *et al*., 2017, 2018). Factors that influence the development of cultivars resistant to Sclerotinia blight include a complex inheritance pattern, plant morphology, the narrow genetic base of cultivated peanut, and available sources of resistance. The inheritance mechanism of host resistance to Sclerotinia blight is not well understood but is known to be quantitative with possible cytoplasmic effects (Wildman *et al*.,1992; 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). Cultivated peanut has an extremely narrow genetic base which has been explained to have resulted from a single domestication event (Simpson *et al*., 2001) and subsequent inbreeding among a few select parental lines in commercial breeding programs. In order to develop new cultivars with enhanced Sclerotinia blight resistance, breeders must search for new sources of resistance outside the cultivated peanut background.

Fortunately, there are peanut germplasm collections globally that contain a wealth of genetic diversity from which breeders can incorporate traits of interest. The largest collections are held by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), the United States Department of Agriculture (USDA), the Oil Crops Research Institute, Chinese Academy of Agricultural Sciences (OCRI-CAAS) and the Empresa

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Table 1. Genotypes tested in this study with known phenotypes along with respective Sclerotinia blight relative scores, field phenotype, marker score, and marker predicted phenotype.

Genotype	Sclerotinia blight relative score[1]	Field Phenotype	Marker Score[2]		Marker Predicted Phenotype[3]
			115 bp band (R)	100 bp band (S)	
ASEM 400	2.1	S	0	1	S
CMP-10	1.8	MS	0	1	MS
EC-98	1.9	MS	0	1	S
Marc 1	1.8	MS	0	1	MS
MDR 98	1.2	MR	1	0	R
Mf10_2308	1.4	MR	0	1	S
Mf10_2927	1.3	MR	1	0	R
Mf10_2943	1.4	MR	0	1	S
Mf10_3040	2.3	S	0	1	S
Tamrun96	2.2	S	0	1	S
Tamrun98	1.4	MR	0	1	MS
Virginia5	1.8	MS	0	1	S
ASEM 484 INTA	1.9	MS	0	1	S
Mf493	1.8	MS	0	1	MS
Víctor ASEM-INTA	2.2	S	0	1	S
Virugard	2.3	S	0	1	S
PI 274193	0.1	R	1	0	R
Tamspan90	1.0	R	1	0	R
Okrun	2.6	S	0	1	S

[1]Relative score based on a 1 to 4 scale, with a score of 0 (no symptoms), 1 (one limb affected), 2 (25% limbs affected), 3 (25-50% limbs affected), or 4 (>50% limbs affected).

[2]Marker score for each band of interest: R = resistant band; S = Susceptible band; 0 = band is not present, 1 = band is present.

[3]Predicted phenotype based on marker profile and peak height (Table 2); [3]R = resistant; MR = moderately resistant; LR = low resistance; MS = moderately susceptible; S = susceptible.

Brasileira de Pesquisa Agropecuaria (EMBRAPA) in Brazil. Other peanut producing countries also maintain smaller collections. These collections are vast and contain thousands of accessions collected from around the world but are difficult to screen in their entirety due to the financial and personnel limitations of most peanut breeding programs. In most cases, a sub-set of these large collections called 'core collections', have been developed and are representative of the genetic diversity available in the complete collection. These core collections, along with the development and use of molecular markers associated with key traits, greatly increase the efficiency of mining these vast collections for sources of breeding material to enhance cultivated peanut. A molecular marker associated with Sclerotinia blight resistance in peanut has been identified (Chenault *et al*., 2009), and has been used to screen multiple core collections (Chamberlin *et al*., 2010; Chamberlin, 2014; Chamberlin and Puppala, 2018).

Argentina ranks 7th world-wide in peanut production (USDA Foreign Agricultural Service, 2020), producing more than 350,000 ha and harvesting approximately 1.1 million tons, annually. The Instituto Nacional de Tecnología Agropecuaria (INTA) Manfredi, Argentina peanut collection consists of 3443 active entries, from 40 countries (although most come from South America). The entries include mostly landraces, but also encompass cultivars and experimental lines. A core collection of 154 entries representing the total genetic variability of the entire collection has been developed (Baldessari *et al*., 2017), but has not yet been screened for sources of Sclerotinia blight resistance. The objective of this study was to characterize the Argentinean INTA core collection using the molecular marker associated with Sclerotinia blight resistance.

Materials and Methods

Genetic Materials. Genomic DNA from 154 accessions from the INTA peanut core collection and 33 supplemental genotypes, many of which had been extensively phenotyped for Sclerotinia blight resistance in Argentina (Table 1), was provided by E. Mamani and V. Moreno of Instituto Nacional de Tecnología Agropecuaria (INTA), Manfredi, Argentina. The DNA extrac-

tion was performed from 20 mg of dried leaves in silica gel. The samples were ground for two 5-s cycles in bead mill at 20,000 rpm (Super FastPrep-2 Bead Beating System, MP Biomedicals LLC, Irvine, CA, USA). A modified CTAB method with a sorbitol cleaning wash before the lysis step was used (Inglis *et al*., 2018). DNA was re-suspended in 100 μL of Tris-EDTA buffer and stored at -20 C until further use.

Phenotyping of Control Genotypes. Phenotyping trials were arranged in a randomized complete-block design with three replications and planted at INTA's Manfredi Experimental Station near Manfredi, (Córdoba Province), Argentina. Individual plots consisted of single rows of 4 m length with a spacing of 1.4 m and planted at a seeding rate of 10 seeds/m. Entries included in these phenotyping trials include those listed in Table 1, with the exception of Okrun (Banks *et al*., 1989) and PI 274193 which were similarly phenotyped in the U.S. Plots were inoculated with 70 cc wheat grain containing active *S. minor* mycelium at approximately 120 d after planting (DAP). Individual plants within a plot were rated in the field after digging according to the following scale: 0 (no symptoms), 1 (one limb affected), 2 (25% limbs affected), 3 (25-50% limbs affected), and 4 (>50% limbs affected). Trials were repeated for 3 yr. Sclerotinia blight relative score was calculated by averaging individual plant scores within each plot.

Marker Analysis. Prior to amplification, DNA was quantified using a NanoDrop nd-3300 spectrofluorometer using the Pico Green dsDNA Assay Kit (ThermoFisher Scientific, Waltham, MA), and concentrations were adjusted to 25 ng/*u*L prior to PCR amplification. Amplification was performed in triplicate using a SSR marker derived from the SSR primer pair pPGPseq2E6, which has been reported to be associated with Sclerotinia blight resistance in peanut (Chenault *et al*., 2009). Amplification was carried out in an Applied Biosystems (Foster City, CA) MiniAmp-Plus thermal cycler under conditions previously optimized. Primers were labelled with 5-FAM fluorophor. Fragment analysis of PCR products was done using an Applied Biosystems (Foster City, CA) 3730 DNA Analyzer and sized using a LIZ120 labelled size standard. Amplification with this primer set generally produces two bands of interest, one at 100 bp (predominant in susceptible genotypes) and one at 115 bp (predominant in resistant genotypes). Peaks of amplified products were analysed using PeakScanner 1.0 software (ThermoFisher Scientific). Peak heights were recorded for each genotype. DNA from known susceptible cultivar control Okrun and known resistant control PI 274193 (USDA Peanut Germplasm Collection) were included in each assay. Genotypes possessing the 115 bp band associated with Sclerotinia blight resistance were given a score of one (1), while those carrying only the 100 bp band were given a zero (0) rating. Genotypes possessing amplified products but neither band of interest were not rated (NR). Genotypes that did not possess any amplified products were not included in the analysis. Correlation analysis of phenotypic and genotypic data was conducted using SAS ver. 9.3 (Cary, NC).

Results and Discussion

In addition to the 154 members of the INTA peanut core collection genotyped in this study, we included a set of 33 other peanut genotypes, many of which had also been phenotyped for Sclerotinia blight resistance in Argentina for 3 yr. Also included in this study were two genotypes (Okrun and PI 274193) that have been similarly genotyped and phenotyped in the U.S., and have been reported previously (Chenault *et al*., 2009; Chamberlin *et al*., 2010; Chamberlin, 2014) to serve as resistant and susceptible controls for this marker analysis. The INTA peanut core collection accessions have been classified by botanical variety and consists of 37% *fastigiata*, 18% *hypogaea*, 13% *peruviana*, 5% *vulgaris*, 5% *aequatoriana*, and 3% *hirsuta* (Figure 1A). Nineteen percent of the accessions have not been botanically classified.

Using the SSR marker previously associated with Sclerotinia blight resistance (Chenault *et al*., 2009), successful amplification was achieved for all but 14 INTA core collection accessions. Lack of amplification for those 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). When comparing genotypic and phenotypic data for the genotypes already phenotyped in the field, the marker predicted phenotype agreed with the phenotyped observed in field trials with few exceptions (Table 1). Linear regression analysis of known phenotypes and genotypic peak height (Figure 2A) indicated there is a correlation between presence of a the 100 bp band with susceptibility to Sclerotinia blight (R^2= 0.7348) and presence of the 115 bp band with resistance (R^2= 0.5059). Genotypic score and resistance level of control phenotyped entries are shown in Figure 2B. Stronger correlations exist for the extremes of

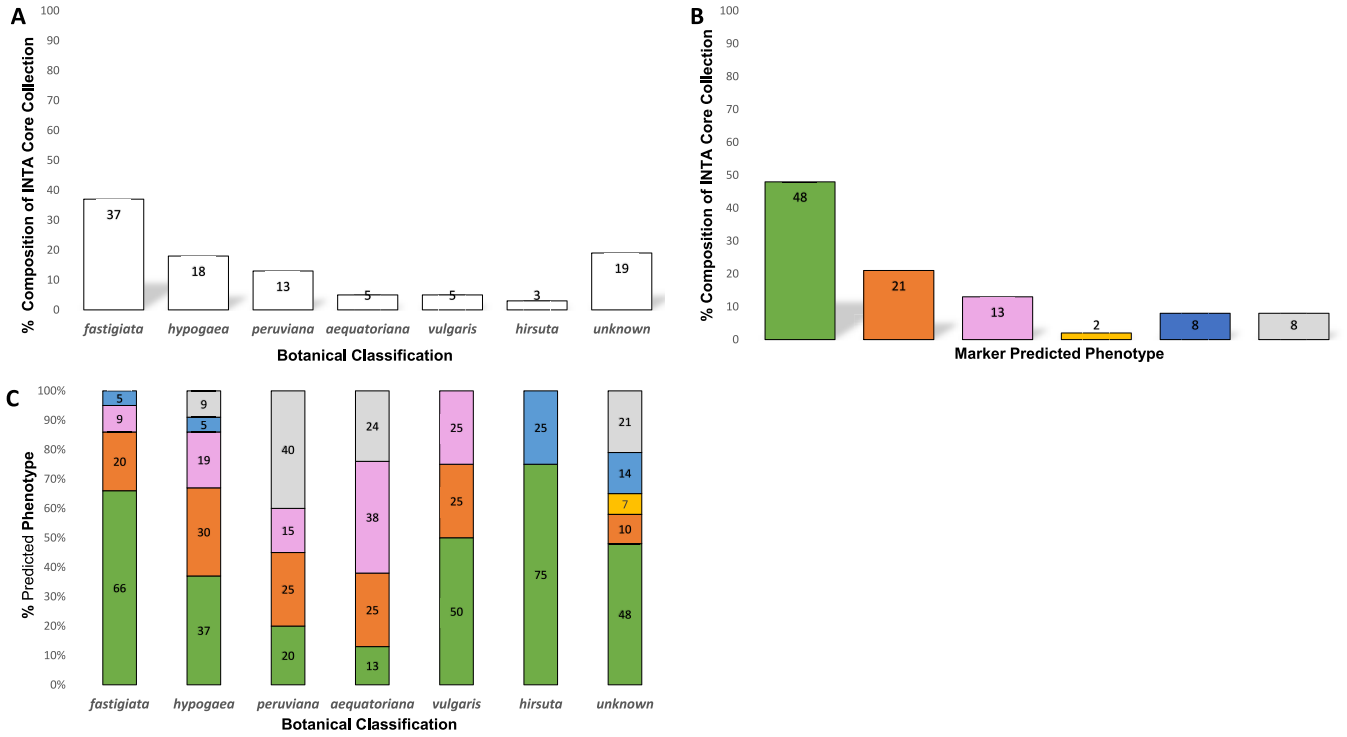

Fig. 1. Classification of the Instituto Nacional de Tecnología Agropecuaria (INTA) core collection, (A) Botanical variety categorization of INTA core collection accessions; (B) Percent composition of INTA core collection by marker predicted phenotype. Green = resistant (R), orange = moderately resistant (MR), pink = low resistance (LR), yellow = moderately susceptible (MS), blue = susceptible (S), grey = not rated (NR); (C) Marker predicted phenotypes of INTA core collection accessions by botanical variety. Green = resistant (R), orange = moderately resistant (MR), pink = low resistance (LR), yellow = moderately susceptible (MS), blue = susceptible (S), grey = not rated (NR).

susceptibility or resistance (Figure 2A and 2B), whereas the marker is less likely to accurately predict phenotypic reactions in the moderate range (denoted by red ovals, Figure 2). These results are similar to those reported previously by others using this marker to screen other germplasm collections (Chamberlin et al, 2010; Chamberlin, 2014; Bennett *et al*., 2018; Chamberlin and Puppala, 2018). Resistance to *S. minor* has been shown to be complex and quantitative (Wildman *et al*., 1992). Resistance genes to Sclerotinia blight are not the only factor influencing disease in peanut. Plant architecture also influences resistance to *S. minor* and other pathogens, with drier open canopies found in *fastigiata* and *vulgaris* botanical-types (Valencia and Spanish market-types, respectively)

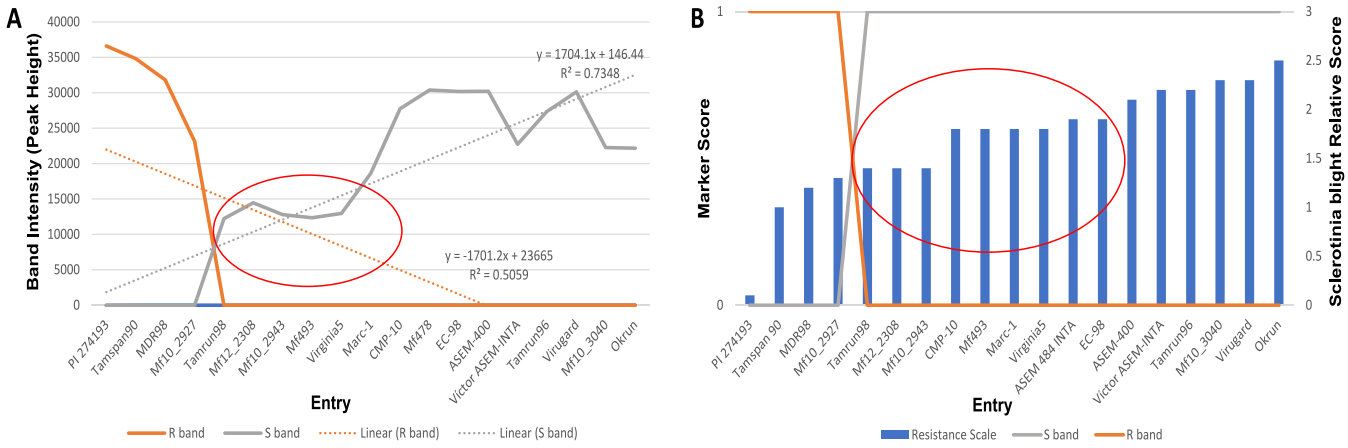

Fig. 2. Correlation of marker profile with resistance of control genotypes. (A) Linear regression analysis of peak height with resistance levels of entries with control phenotypes. Red oval denotes region of uncertainty for marker predicted phenotypes; (B) Genotypic score and resistance levels of entries with control phenotypes. Red oval denotes region of uncertainty for marker predicted phenotypes.

being less favorable to disease than the more humid and dense canopies of the *hypogaea* botanical-types (runner and Virginia market-types, respectively) (Coffelt and Porter, 1982; Chappell *et al*., 1995; Goldman *et al*., 1995). The interaction between growth habit and Sclerotinia blight resistance on disease incidence may account for the exceptions in the marker analysis.

Given that the template DNA has been quantified and normalized across all reactions, band intensity or peak height of the identified resistance and susceptible bands has previously been correlated with level of resistance seen among genotypes tested with this marker system (Chamberlin, 2014; Chamberlin and Puppala, 2018), allowing the prediction of field reaction to *S. minor* infection for genotypes not yet tested in phenotyping trials. Table 2 lists the botanical variety and genotypic profiles (peak heights) for each entry along with the respective predicted field reaction to *S. minor*. Of the 154 core accessions tested, the marker predicted phenotype (Figure 1B) was 48% resistant (R), 21% moderately resistant (MR), 13% low resistant (LR), 2% moderately susceptible (MS), and 8% susceptible (S). Eight percent of the accessions did not produce a marker genotype and thus were not rated (NR) for predicted phenotype. The predicted phenotypes by botanical variety group (Figure1C) were as follows: *fastigiata* 66% R, 20% MR, 9% LR and 5% S; *hypogaea* 37% R, 30% MR, 15% LR, 5% S and 9% NR; *peruviana* 20% R, 25% MR, 15% LR, 40% NR; *aequatoriana* 13%R, 25% MR, 38% LR, 24% NR; *hirsuta* 75% R, 25% S; *vulgaris* 50% R, 25% MR, 25% LR.

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). The marker has been used previously to screen numerous germplasm collections (Chamberlin et al, 2010; Chamberlin, 2014; Chamberlin and Puppala, 2018) and the results obtained have been shown to closely agree with phenotypic reaction to *S. minor* in field trials (Chenault et al, 2009; Chamberlin *et al*., 2010; Damicone *et al*., 2010; Bennett *et al*., 2018). The results of the current study are similar to those reported when the marker was used to screen other collections, with the marker for resistance being prominent in the *fastigiata*, *vulgaris,* and *hirsuta* botanical types.

Markers closely associated with phenotypic traits can be used to increase screening efficiency when working with large germplasm collections. Such markers can also be used to track inheritance of traits while developing new cultivars. Although primitive compared with some other crops, markers have been identified in peanut for resistance to bacterial wilt (Ren *et al*., 2008), rust (Hou *et al*., 2007; Shoba *et al*., 2010), late leaf spot (Xia *et al*., 2007; Shoba *et al*., 2010), root-knot nematode (Chu *et al*., 2007), and *Aspergillus flavus* (Lei *et al*., 2005). The marker used in this study was used to identify PI 274193 of the USDA peanut germplasm collection as a source of excellent resistance to Sclerotinia blight (Damicone *et al*., 2010; Chamberlin *et al*., 2010) which was then incorporated into a cultivated background, leading to the development of the cultivar Lariat (Chamberlin *et al*., 2018), which requires no fungicide treatment for Sclerotinia blight control.

Keeping in mind that molecular markers, including the one used in this study, cannot accurately predict the level of disease resistance that will be demonstrated in field trials, use of molecular markers should be used as a tool only to screen vast collections and identify germplasm with the potential of field resistance. The results obtained in this study have identified accessions from the INTA core collection worthy of evaluation in the field for *S. minor* resistance and identified those with low probability of being a source of resistance, reducing the amount of field work required to select from the collection by 52%. Accessions within each botanical variety represented in the Argentinean INTA core collection worthy of field testing have been identified which may assist in defining breeder choices when designing cultivar development. Although screening for traits with molecular markers cannot necessarily predict the level of high disease resistance that will be demonstrated in the field and will not replace the breeder's eye in cultivar development, the reduction in resources required to screen large collections of germplasm for a trait of interest will ultimately increase the breeder's efficiency and reduce the time required for improved cultivar release.

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Table 2. Argentinean Instituto Nacional de Tecnología Agropecuaria (INTA) core collection accessions and control genotypes tested in this study, along with botanical variety, marker band peak heights and marker predicted phenotypes.

Genotype	Botanical Variety[1]	100 bp	115 bp	Predicted Phenotype[2]	Genotype	Botanical Variety[1]	100 bp	115 bp	Predicted Phenotype[2]	Genotype	Botanical Variety[1]	100 bp	115 bp	Predicted Phenotype[2]
REM204	F		23946	R	REM2397	A		29091	R	REM3839	P	7063		MS
REM333	F		8415	MR	REM2400	A		2216	LR	REM3843	P		24113	R
REM508	F		29605	R	REM2466	P		30395	R	REM3854	P			NR
REM525	H			NR	REM2469	P			NR	REM3911	H	27132		S
REM543	H	9362		MS	REM2474	F		30606	R	REM4116	U		26043	R
REM547	H		29315	R	REM2485	P			NR	REM4118	U		23820	R
REM548	H		9053	LR	REM2534	H		16698	MR	REM4172	F		14483	MR
REM549	H		29831	R	REM2535	U		27952	R	REM4333	U		19488	MR
REM552	H		17315	MR	REM2673	U		17715	MR	REM4336	V		23298	R
REM556	H		14227	MR	REM2696	U	27448		S	REM4562	U		27924	R
REM591	H		31534	R	REM2698	U	12180		MS	REM4584	H	23557		S
REM598	H		25504	R	REM2708	U	26756		S	REM4902	U		24737	R
REM609	H		15346	MR	REM2727	U	21609		S	REM4906	U		28409	R
REM610	H		25328	R	REM2749	U	18610		MS	REM4908	U	23388		S
REM611	H		31273	R	REM2884	U		27675	R	REM4909	U	21391		S
REM633	H	27607		S	REM2885	R	22222		S	REM4911	U	29743		S
REM664	H		30964	R	REM2919	R		21836	R	REM4913	U	26652		S
REM686	H		6724	LR	REM2920	F		23070	R	REM4914	U	29617		S
REM695	H		23409	R	REM2941	F		26928	R	REM4915	F			NR
REM722	H		31524	R	REM2948	V		14799	MR	REM4916	P			NR
REM843	H		1601	LR	REM2949	V		28721	R	REM5133	U		26965	R
REM874	H		12619	MR	REM2955	V		29496	R	REM5170	U		28173	R
REM1006	H		8151	LR	REM2960	V		21182	R	REM5272	F		28202	R
REM1037	H		24368	R	REM2961	V		5339	LR	REM5354	U		27631	R
REM1045	H		3659	LR	REM2995	F		21924	R	REM6026	U		19995	MR
REM1046	H		19402	MR	REM3014	F		12675	MR	REM6087	A		21450	MR
REM1047	F		26791	R	REM3018	F		24363	R	REM6101	U			NR
REM1048	F		30531	R	REM3023	F		28465	R	REM6140	A			NR
REM1181	F		12941	MR	REM3025	F		27473	R	REM6156	U			NR
REM1184	P		11620	MR	REM3042	F		27621	R	REM6158	A		17536	MR
REM1186	P		15734	MR	REM3045	F		20221	R	Ascasubi H.	V		20710	R
REM1197	F			NR	REM3046	F		21160	R	Pronto	V			NR
REM1210	U			NR	REM3049	F		28868	R	Tamrun OL01	H			NR
REM1224	F		31164	R	REM3052	F		28369	R	Granoleico	H	29186		S
REM1251	F		28197	R	REM3174	F		25580	R	GA-06G	H	29790		S
REM1503	F		24653	R	REM3264	F		19426	MR	Colorado I.	F		13440	MR
REM1513	F		17898	MR	REM3272	F		25776	R	Mf5_1655	H	27545		S
REM1520	F		16038	MR	REM3280	F		25501	R	Mf10_2870	H	27786		S
REM1525	F		19111	MR	REM3288	F		26216	R	Tamspan 90	V		14826	MR
REM1528	F		27184	R	REM3298	F		26143	R	Mf10_3255	H	28622		S
REM1537	F		12748	MR	REM3318	V		19186	MR	Mf478	V	30379		S

Table 2. Continued.

Genotype	Botanical Variety[1]	100 bp	115 bp	Predicted Phenotype[2]	Genotype	Botanical Variety[1]	100 bp	115 bp	Predicted Phenotype[2]	Genotype	Botanical Variety[1]	100 bp	115 bp	Predicted Phenotype[2]
REM1538	F		24650	R	REM3323	F		24529	R	ASEM 484	H	28548		S
REM1540	F		14096	MR	REM3339	V		3944	LR	Mf489	H	1373		MS
REM1550	F		27274	R	REM3373	U		24594	R	Fla. MDR98	H		21262	R
REM1554	F		16632	MR	REM3393	F		26028	R	Mf493	H	12350		MS
REM1560	F	9085		MS	REM3602	H		10070	MR	HSCP89-2	H		28300	R
REM1562	F		2430	LR	REM3606	H		17205	MR	Víctor ASEM	H	2730		MS
REM1579	F		7614	LR	REM3617	R		27479	R	Virugard	H	30123		S
REM1596	F		8536	LR	REM3618	R		25044	R	ASEM 400	H	30225		S
REM1609	F		28666	R	REM3619	F		23479	R	Mf10_2927	H		23129	R
REM1614	F		8810	LR	REM3693	F		23968	R	Tamrun 98	H	16223		MS
REM1615	F		12946	MR	REM3703	P		5684	LR	Mf12_2308	H	24463		S
REM1725	F		27088	R	REM3715	P		18714	MR	Mf10_2943	H	30775		S
REM1734	F		27835	R	REM3729	P			NR	C-99R	H			NR
REM1771	F		29690	R	REM3733	P		15615	MR	CMP-10	H	7735		MS
REM1844	F		20553	R	REM3736	P		17468	MR	Marc-1	H	18629		MS
REM1862	F		28937	R	REM3748	P			NR	Virginia 5	H	29954		S
REM2038	U		26684	R	REM3760	P		27341	R	EC-98	H	30170		S
REM2056	U		21285	R	REM3766	P			NR	Tamrun 96	H	27356		S
REM2357	A			NR	REM3809	P			NR	Mf10_3040	H	22261		S
REM2367	A		1274	LR	REM3815	P		2960	LR	PI 274193	H		36604	R
REM2394	A		6475	LR	REM3829	P		29698	R	Okrun	H	22180		S

[1]Botanical variety: F = *fastigiata*; P = *peruviana*; H = *hypogaea*; V = *vulgaris*; R = *hirsuta*; A = *aequatoriana*, U = unknown

[2]Marker predicted phenotype: R = resistant; MR = moderately resistant; LR = low resistance; MS = moderately susceptible; S = susceptible; NR = not rated

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