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Molecular diagnosis to identify new sources of resistance to sclerotinia blight in groundnut (*Arachis hypogaea* L.)

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Abstract Sclerotinia blight, caused by soil-borne fungus *Sclerotinia minor* Jagger, is one of the destructive diseases in groundnut. Pathogen affected plants usually displays lesions, wilt and collapse which cause high yield losses. Traditional field screening is time and resources consuming. Molecular markers associated with resistance genes offer an alternative selection technique which is relatively easy, more definite and not influenced by environmental fluctuations. In the present investigation, a marker-assisted diagnosis was done to screen 256 diverse germplasm for the presence or absence of SSR markers reported resistance or susceptibility to sclerotinia blight. One hundred and forty two genotypes from different botanical varieties were recognized as new potential sources of resistance to sclerotinia blight for field evaluation. The banding pattern related to the disease resistance is observed at high frequency in the variety *vulgaris* (39.4 %) and less distributed in the varieties *fastigiata* (38.0 %) and *hypogaea* (19.7 %) among the resistant genotypes in the collection. These genotypes had same banding pattern as reported for resistance germplasm. This work reports the successful application of marker-assisted diagnosis as a tool to identify resistance to sclerotinia blight in diverse collections.

Keywords *Sclerotinia minor* Jagger · Disease resistance · Pathogen · Molecular markers · Marker-assisted selection

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Introduction

Groundnut (*Arachis hypogaea* L., Fabaceae), also known as peanut, is one of the major oilseed crops worldwide in tropical, subtropical and warm areas of approximately 100 countries, grown on 24.7 million ha with a total production of nearly 41 million tons annually (FAO 2012). About two-thirds of global production is used for vegetable oil and the remaining is utilized for edible product and as seed (Upadhyaya et al. 2011). Groundnut is a rich source of edible oil (about 48 %) and protein (about 26 %), used globally for human nutrition (Sarvamangala et al. 2011). The biological value of groundnut protein is among the highest of the vegetable proteins (Shoba et al. 2012). The cake obtained after extraction of oil and plant haulms are used in the livestock feed industry (Nigam and Aruna 2008). Groundnut also fixes atmospheric nitrogen, thus, improving the soil fertility.

Many biotic and abiotic stress factors limit the groundnut production in various eco-agricultural

systems. Leaf spots, rust and soil-borne diseases among the biotic stresses are globally important diseases affecting both production and seed quality (Liao and Holbrook 2007). Of recent, soil-borne pathogens have become more important in many parts of the world (Thiessen and Woodward 2012). Sclerotinia blight is one of the most destructive soil-borne diseases of groundnut (Livingstone et al. 2005), causing yield losses up to 50 % (Butzler et al. 1998). Sclerotinia blight is caused by soil-borne fungus *S. minor* Jagger which is an ascomycetes that produce white aerial mycelia and black, irregularly shaped sclerotia (Thiessen and Woodward 2012). Soil with pH near 6.5, cool temperatures and high relative humidity or rainfall are the favorable conditions for sclerotial germination and infection (Bailey and Brune 1997). Pathogens usually attack groundnut root and stem at or near the soil surface and destroy the vascular tissue of the crown, at which time the plant wilts and collapses (Laemmlen 2001). Several fungicides have been recommended to control the disease (Smith et al. 1992). Cultural practices such as crop rotation (Melouk and Backman 1995), tillage practices (Wu and Subbarao 2003), seed fungicide-treatment (Porter and Melouk 1997), and drip irrigation have been used to reduce the production costs, and soil contamination by fungicides (Gil et al. 2008) but these measures have not been sufficient to disease control. Host plant resistance is the most effective solution to manage sclerotinia blight and protect the environment (Chenault et al. 2009). However, desired progress through genetic resistance has not been achieved because of complicated inheritance mechanism and limited number of known sources of resistance (Chamberlin et al. 2010). Screening of more germplasm might help to find new sources of resistance to soil borne diseases to support breeding programs.

Traditional method of screening of germplasm in affected field plots is time and resource consuming. Several factors contribute to the development of uniform occurrence of diseases in the field conditions, which often make it difficult to achieve uniform infestation of disease pressure on test genotypes, leading to misclassification of germplasm. Advances in molecular marker technologies have opened the door to applying these techniques for screening of breeding populations to increase the efficiency of selection (Boopathi 2013). Marker assisted diagnosis probably would be cost-effective and faster for selection of resistance vis-à-vis field evaluations (Arus and Moreno-Gonzalez 1993; Ender et al. 2008; Ashkani et al. 2012). Simple sequence repeats (SSRs) are the marker of choice for use in such applications as these are reproducible and co-dominant markers used extensively in breeding studies (Mondal and Badigannavar 2010). Simple sequence repeats in groundnut have been used to identify resistance to late leaf spot (Shoba et al. 2012), rust (Mace et al. 2006), and nematode (Nagy et al. 2010). Simple sequence repeats associated with resistance to sclerotinia blight have been reported in groundnut (Chenault et al. 2009), which were used to characterize U.S. peanut mini core collection (Chamberlin et al. 2010) to identify sclerotinia blight resistant germplasm.

The aim of this investigation was to identify new sources of variation for sclerotinia blight in 256 peanut germplasm using SSR markers previously reported associated with resistance to sclerotinia blight.

Materials and methods

Plant materials

Two hundred and fifty six groundnut germplasm, which include ICRISAT groundnut mini core collection (Upadhyaya et al. 2002), breeding lines, local landraces, and registered cultivars, were used as a genetic material in this study (Table 1). PI 482189 (resistant) and PI 496448 (susceptible) were used as controls by Chamberlin et al. (2010) to differentiate the test materials into resistance or susceptible category based on SSR marker profile.

Molecular analysis

The seeds were germinated in the West Mediterranean Agricultural Research Institute's fields of Antalya, Turkey (36°52′N. 30°50′E. 15 m elevation). Groundnut leaves were collected from plants and stored at −80 °C for DNA extraction. DNA isolation was carried out using the CTAB method (Doyle and Doyle 1990). The quality and quantity of the DNA extracts were checked by agarose gel electrophoresis with a DNA standard. The DNA extracts were suspended in milli-Q PCR water and stored at −20 °C.

The PCR analyses were conducted and templates for PCR reaction set up for 20 µl as follows: 2 µl of

Table 1 Association of sclerotinia blight resistant marker with the genotypes of groundnut collection

Accession No.	ICRISAT Genebank Entry (ICG) / Cultivar Name	Subspecies	Botanical variety	Marker Score*	Accession No.	ICRISAT Genebank Entry (ICG) / Cultivar Name	Subspecies	Botanical variety	Marker Score
ACG 1	ICG 36	*fastigiata*	*vulgaris*	L	ACG 56	ICG 4412	*hypogaea*	*hypogaea*	S
ACG 2	ICG 76	*hypogaea*	*hypogaea*	S	ACG 57	ICG 4527	*hypogaea*	*hypogaea*	S
ACG 3	ICG 81	*fastigiata*	*vulgaris*	L	ACG 58	ICG 4538	*hypogaea*	*hypogaea*	S
ACG 4	ICG 111	*hypogaea*	*hypogaea*	S	ACG 59	ICG 4543	*fastigiata*	*vulgaris*	L
ACG 5	ICG 115	*fastigiata*	*fastigiata*	L	ACG 60	ICG 4598	*hypogaea*	*hypogaea*	S
ACG 6	ICG 118	*fastigiata*	*vulgaris*	S	ACG 61	ICG 4670	*fastigiata*	*fastigiata*	L
ACG 7	ICG 156	*hypogaea*	*hypogaea*	S	ACG 62	ICG 4684	*fastigiata*	*vulgaris*	L
ACG 8	ICG 163	*hypogaea*	*hypogaea*	S	ACG 63	ICG 4729	*fastigiata*	*vulgaris*	L
ACG 9	ICG 188	*hypogaea*	*hypogaea*	L	ACG 64	ICG 4746	*hypogaea*	*hypogaea*	L
ACG 10	ICG 297	*fastigiata*	*fastigiata*	L	ACG 65	ICG 4750	*fastigiata*	*vulgaris*	L
ACG 11	ICG 332	*fastigiata*	*fastigiata*	L	ACG 66	ICG 4911	*fastigiata*	*vulgaris*	L
ACG 12	ICG 334	*fastigiata*	*vulgaris*	L	ACG 67	ICG 4955	*fastigiata*	*vulgaris*	L
ACG 13	ICG 397	*fastigiata*	*fastigiata*	L	ACG 68	ICG 4998	*hypogaea*	*hypogaea*	S
ACG 14	ICG 434	*fastigiata*	*vulgaris*	L	ACG 69	ICG 5016	*hypogaea*	*hypogaea*	S
ACG 15	ICG 442	*fastigiata*	*vulgaris*	L	ACG 70	ICG 5195	*fastigiata*	*vulgaris*	L
ACG 16	ICG 513	*hypogaea*	*hypogaea*	S	ACG 71	ICG 5221	*fastigiata*	*fastigiata*	L
ACG 17	ICG 532	*hypogaea*	*hypogaea*	S	ACG 72	ICG 5236	*fastigiata*	*vulgaris*	L
ACG 18	ICG 721	*hypogaea*	*hypogaea*	S	ACG 73	ICG 5286	*hypogaea*	*hypogaea*	L
ACG 19	ICG 862	*hypogaea*	*hypogaea*	S	ACG 74	ICG 5327	*hypogaea*	*hypogaea*	S
ACG 20	ICG 875	*hypogaea*	*hypogaea*	S	ACG 75	ICG 5475	*fastigiata*	*fastigiata*	L
ACG 21	ICG 928	*hypogaea*	*hypogaea*	S	ACG 76	ICG 5494	*fastigiata*	*vulgaris*	L
ACG 22	ICG 1137	*fastigiata*	*vulgaris*	L	ACG 77	ICG 5609	*fastigiata*	*fastigiata*	L
ACG 23	ICG 1142	*fastigiata*	*fastigiata*	L	ACG 78	ICG 5662	*hypogaea*	*hypogaea*	S
ACG 24	ICG 1274	*fastigiata*	*fastigiata*	L	ACG 79	ICG 5663	*hypogaea*	*hypogaea*	S
ACG 25	ICG 1399	*fastigiata*	*fastigiata*	L	ACG 80	ICG 5745	*hypogaea*	*hypogaea*	S
ACG 26	ICG 1415	*fastigiata*	*vulgaris*	L	ACG 81	ICG 5779	*fastigiata*	*vulgaris*	L
ACG 27	ICG 1519	*fastigiata*	*vulgaris*	L	ACG 82	ICG 5827	*hypogaea*	*hypogaea*	L
ACG 28	ICG 1668	*hypogaea*	*hypogaea*	S	ACG 83	ICG 5891	*hypogaea*	*hypogaea*	S
ACG 29	ICG 1711	*fastigiata*	*vulgaris*	L	ACG 84	ICG 6022	*fastigiata*	*fastigiata*	L
ACG 30	ICG 1973	*fastigiata*	*vulgaris*	L	ACG 85	ICG 6057	*hypogaea*	*hypogaea*	S
ACG 31	ICG 2019	*fastigiata*	*vulgaris*	L	ACG 86	ICG 6201	*fastigiata*	*fastigiata*	L
ACG 32	ICG 2106	*fastigiata*	*vulgaris*	L	ACG 87	ICG 6263	*fastigiata*	*vulgaris*	L
ACG 33	ICG 2381	*hypogaea*	*hypogaea*	L	ACG 88	ICG 6375	*fastigiata*	*vulgaris*	L
ACG 34	ICG 2511	*hypogaea*	*hypogaea*	S	ACG 89	ICG 6402	*hypogaea*	*hypogaea*	L
ACG 35	ICG 2738	*fastigiata*	*fastigiata*	L	ACG 90	ICG 6407	*fastigiata*	*vulgaris*	L
ACG 36	ICG 2772	*hypogaea*	*hypogaea*	S	ACG 91	ICG 6646	*fastigiata*	*fastigiata*	N/A
ACG 37	ICG 2773	*hypogaea*	*hypogaea*	L	ACG 92	ICG 6654	*fastigiata*	*vulgaris*	N/A
ACG 38	ICG 2777	*hypogaea*	*hypogaea*	S	ACG 93	ICG 6667	*hypogaea*	*hypogaea*	S
ACG 39	ICG 2857	*hypogaea*	*hypogaea*	L	ACG 94	ICG 6703	*hypogaea*	*hypogaea*	L
ACG 40	ICG 2925	*hypogaea*	*hypogaea*	S	ACG 95	ICG 6766	*hypogaea*	*hypogaea*	S
ACG 41	ICG 3027	*hypogaea*	*hypogaea*	S	ACG 96	ICG 6813	*hypogaea*	*hypogaea*	S
ACG 42	ICG 3053	*hypogaea*	*hypogaea*	S	ACG 97	ICG 6888	*fastigiata*	*fastigiata*	L
ACG 43	ICG 3102	*fastigiata*	*vulgaris*	L	ACG 98	ICG 6892	*hypogaea*	*hypogaea*	S
ACG 44	ICG 3240	*fastigiata*	*vulgaris*	L	ACG 99	ICG 6993	*hypogaea*	*hypogaea*	L
ACG 45	ICG 3343	*fastigiata*	*vulgaris*	L	ACG 100	ICG 7000	*hypogaea*	*hypogaea*	L
ACG 46	ICG 3421	*fastigiata*	*vulgaris*	L	ACG 101	ICG 7153	*hypogaea*	*hypogaea*	S
ACG 47	ICG 3584	*fastigiata*	*vulgaris*	L	ACG 102	ICG 7181	*fastigiata*	*fastigiata*	L
ACG 48	ICG 3673	*fastigiata*	*fastigiata*	L	ACG 103	ICG 7190	*fastigiata*	*vulgaris*	L
ACG 49	ICG 3681	*fastigiata*	*fastigiata*	L	ACG 104	ICG 7243	*hypogaea*	*hypogaea*	S
ACG 50	ICG 3746	*fastigiata*	*vulgaris*	L	ACG 105	ICG 7906	*fastigiata*	*vulgaris*	L
ACG 51	ICG 3775	*fastigiata*	*vulgaris*	L	ACG 106	ICG 7963	*hypogaea*	*hypogaea*	L
ACG 52	ICG 3992	*hypogaea*	*hypogaea*	S	ACG 107	ICG 7969	*fastigiata*	*vulgaris*	L
ACG 53	ICG 4156	*hypogaea*	*hypogaea*	S	ACG 108	ICG 8083	*fastigiata*	*vulgaris*	L
ACG 54	ICG 4343	*hypogaea*	*hypogaea*	S	ACG 109	ICG 8106	*fastigiata*	*fastigiata*	L
ACG 55	ICG 4389	*hypogaea*	*hypogaea*	S	ACG 110	ICG 8285	*hypogaea*	*hypogaea*	S

Table 1 continued

Accession No.	ICRISAT Genebank Entry (ICG) / Cultivar Name	Subspecies	Botanical variety	Marker Score*	Accession No.	ICRISAT Genebank Entry (ICG) / Cultivar Name	Subspecies	Botanical variety	Marker Score
ACG 111	ICG 8490	*hypogaea*	*hypogaea*	S	ACG 166	ICG 13858	*fastigiata*	*fastigiata*	L
ACG 112	ICG 8517	*fastigiata*	*fastigiata*	L	ACG 167	ICG 13941	*fastigiata*	*vulgaris*	S
ACG 113	ICG 8567	*fastigiata*	*vulgaris*	L	ACG 168	ICG 13942	*hypogaea*	*hypogaea*	S
ACG 114	ICG 8760	*hypogaea*	*hypogaea*	S	ACG 169	ICG 13982	*hypogaea*	*hypogaea*	L
ACG 115	ICG 9037	*hypogaea*	*hypogaea*	S	ACG 170	ICG 14008	*hypogaea*	*hypogaea*	S
ACG 116	ICG 9157	*fastigiata*	*vulgaris*	L	ACG 171	ICG 14106	*fastigiata*	*fastigiata*	L
ACG 117	ICG 9249	*fastigiata*	*vulgaris*	L	ACG 172	ICG 14118	*fastigiata*	*vulgaris*	L
ACG 118	ICG 9315	*fastigiata*	*fastigiata*	L	ACG 173	ICG 14127	*fastigiata*	*fastigiata*	L
ACG 119	ICG 9418	*fastigiata*	*vulgaris*	L	ACG 174	ICG 14466	*hypogaea*	*hypogaea*	S
ACG 120	ICG 9507	*fastigiata*	*vulgaris*	S	ACG 175	ICG 14475	*hypogaea*	*hypogaea*	L
ACG 121	ICG 9666	*hypogaea*	*hypogaea*	S	ACG 176	ICG 14482	*hypogaea*	*hypogaea*	L
ACG 122	ICG 9777	*hypogaea*	*hypogaea*	S	ACG 177	ICG 14523	*hypogaea*	*hypogaea*	S
ACG 123	ICG 9809	*fastigiata*	*vulgaris*	L	ACG 178	ICG 14630	*fastigiata*	*fastigiata*	L
ACG 124	ICG 9842	*hypogaea*	*hypogaea*	S	ACG 179	ICG 14705	*hypogaea*	*hypogaea*	L
ACG 125	ICG 9905	*hypogaea*	*hypogaea*	S	ACG 180	ICG 14710	*fastigiata*	*fastigiata*	L
ACG 126	ICG 9961	*hypogaea*	*hypogaea*	S	ACG 181	ICG 14985	*fastigiata*	*vulgaris*	L
ACG 127	ICG 10036	*fastigiata*	*peruviana*	L	ACG 182	ICG 15042	*fastigiata*	*fastigiata*	L
ACG 128	ICG 10092	*fastigiata*	*fastigiata*	L	ACG 183	ICG 15190	*hypogaea*	*hypogaea*	S
ACG 129	ICG 10185	*hypogaea*	*hypogaea*	S	ACG 184	ICG 15287	*fastigiata*	*vulgaris*	L
ACG 130	ICG 10384	*fastigiata*	*vulgaris*	L	ACG 185	ICG 15309	*fastigiata*	*fastigiata*	L
ACG 131	ICG 10474	*fastigiata*	*fastigiata*	L	ACG 186	ICG 15419	*hypogaea*	*hirsuta*	L
ACG 132	ICG 10479	*hypogaea*	*hypogaea*	L	ACG 187	NC-7	*hypogaea*	*hypogaea*	S
ACG 133	ICG 10554	*fastigiata*	*fastigiata*	L	ACG 188	PF-259860	*hypogaea*	*hypogaea*	S
ACG 134	ICG 10566	*fastigiata*	*fastigiata*	L	ACG 189	NC-3033	*hypogaea*	*hypogaea*	S
ACG 135	ICG 10890	*fastigiata*	*fastigiata*	L	ACG 190	5015	*hypogaea*	*hypogaea*	S
ACG 136	ICG 11088	*fastigiata*	*peruviana*	L	ACG 191	5026	*hypogaea*	*hypogaea*	S
ACG 137	ICG 11109	*hypogaea*	*hypogaea*	L	ACG 192	5030	*hypogaea*	*hypogaea*	L
ACG 138	ICG 11144	*fastigiata*	*fastigiata*	L	ACG 193	5067	*hypogaea*	*hypogaea*	L
ACG 139	ICG 11219	*hypogaea*	*hypogaea*	S	ACG 194	88/3	*hypogaea*	*hypogaea*	S
ACG 140	ICG 11249	*fastigiata*	*vulgaris*	L	ACG 195	Ant-92/1	*hypogaea*	*hypogaea*	S
ACG 141	ICG 11322	*hypogaea*	*hypogaea*	S	ACG 196	427-24	*hypogaea*	*hypogaea*	S
ACG 142	ICG 11426	*hypogaea*	*hypogaea*	L	ACG 197	437-3-4-B-2	*hypogaea*	*hypogaea*	L
ACG 143	ICG 11457	*hypogaea*	*hypogaea*	S	ACG 198	393-2-1-2-2	*hypogaea*	*hypogaea*	N/A
ACG 144	ICG 11515	*fastigiata*	*vulgaris*	L	ACG 199	70/1145-1/03	*hypogaea*	*hypogaea*	L
ACG 145	ICG 11651	*fastigiata*	*vulgaris*	L	ACG 200	75/1073-A	*hypogaea*	*hypogaea*	S
ACG 146	ICG 11687	*fastigiata*	*vulgaris*	L	ACG 201	75/1073-B	*hypogaea*	*hypogaea*	S
ACG 147	ICG 11855	*hypogaea*	*hypogaea*	S	ACG 202	Bari-89	*hypogaea*	*hypogaea*	S
ACG 148	ICG 11862	*hypogaea*	*hypogaea*	S	ACG 203	Best Dagar	*hypogaea*	*hypogaea*	N/A
ACG 149	ICG 12000	*hypogaea*	*hypogaea*	S	ACG 204	V.Banbim P.	*hypogaea*	*hypogaea*	S
ACG 150	ICG 12189	*fastigiata*	*vulgaris*	L	ACG 205	88 Bocounba	*hypogaea*	*hypogaea*	S
ACG 151	ICG 12276	*hypogaea*	*hypogaea*	L	ACG 206	Home bay	*hypogaea*	*hypogaea*	S
ACG 152	ICG 12370	*hypogaea*	*hypogaea*	S	ACG 207	Florigiant	*hypogaea*	*hypogaea*	L
ACG 153	ICG 12625	*fastigiata*	*aequatoriana*	L	ACG 208	Flamingo	*hypogaea*	*hypogaea*	S
ACG 154	ICG 12672	*hypogaea*	*hypogaea*	S	ACG 209	Shulamit	*hypogaea*	*hypogaea*	S
ACG 155	ICG 12682	*fastigiata*	*vulgaris*	L	ACG 210	Sunrunner	*hypogaea*	*hypogaea*	S
ACG 156	ICG 12697	*fastigiata*	*vulgaris*	N/A	ACG 211	Florunner	*hypogaea*	*hypogaea*	S
ACG 157	ICG 12879	*fastigiata*	*vulgaris*	L	ACG 212	Swallow	*hypogaea*	*hypogaea*	S
ACG 158	ICG 12921	*fastigiata*	*vulgaris*	L	ACG 213	Behirim	*hypogaea*	*hypogaea*	L
ACG 159	ICG 12988	*fastigiata*	*vulgaris*	L	ACG 214	Cine	*hypogaea*	*hypogaea*	S
ACG 160	ICG 13099	*hypogaea*	*hypogaea*	S	ACG 215	Kadriye	*hypogaea*	*hypogaea*	S
ACG 161	ICG 13491	*fastigiata*	*vulgaris*	L	ACG 216	Osmaniye	*hypogaea*	*hypogaea*	N/A
ACG 162	ICG 13603	*fastigiata*	*vulgaris*	L	ACG 217	Osm. Erzin	*hypogaea*	*hypogaea*	S
ACG 163	ICG 13723	*hypogaea*	*hypogaea*	S	ACG 218	Anamur-B	*hypogaea*	*hypogaea*	S
ACG 164	ICG 13787	*hypogaea*	*hypogaea*	S	ACG 219	Anamur-K	*hypogaea*	*hypogaea*	S
ACG 165	ICG 13856	*fastigiata*	*fastigiata*	L	ACG 220	Gazipasa	*hypogaea*	*hypogaea*	S

Table 1 continued

Accession No.	ICRISAT Genebank Entry (ICG) / Cultivar Name	Subspecies	Botanical variety	Marker Score*	Accession No.	ICRISAT Genebank Entry (ICG) / Cultivar Name	Subspecies	Botanical variety	Marker Score
ACG 221	Çom	*hypogaea*	*hypogaea*	S	ACG 239	Schwarz	*fastigiata*	*fastigiata*	L
ACG 222	NC-Fla-14	*hypogaea*	*hypogaea*	S	ACG 240	Spancross	*fastigiata*	*fastigiata*	L
ACG 223	NC-10-C	*hypogaea*	*hypogaea*	S	ACG 241	PF-161317	*fastigiata*	*fastigiata*	L
ACG 224	GP-NC-343	*hypogaea*	*hypogaea*	S	ACG 242	PF-248759	*fastigiata*	*fastigiata*	L
ACG 225	88488	*hypogaea*	*hypogaea*	S	ACG 243	PF-268771-B	*fastigiata*	*fastigiata*	L
ACG 226	88121	*hypogaea*	*hypogaea*	S	ACG 244	C 1-27	*fastigiata*	*fastigiata*	L
ACG 227	PI-315633	*hypogaea*	*hypogaea*	S	ACG 245	AF-2B Grif	*fastigiata*	*fastigiata*	L
ACG 228	PI-315621	*hypogaea*	*hypogaea*	S	ACG 246	Argentine	*fastigiata*	*fastigiata*	L
ACG 229	Edirne-9p-53	*hypogaea*	*hypogaea*	S	ACG 247	Bayramic	*fastigiata*	*fastigiata*	L
ACG 230	M-44-A	*hypogaea*	*hypogaea*	S	ACG 248	Comet	*fastigiata*	*fastigiata*	L
ACG 231	M-44-B	*hypogaea*	*hypogaea*	L	ACG 249	N. M.Valan	*fastigiata*	*fastigiata*	L
ACG 232	Anamur-2006	*hypogaea*	*hypogaea*	L	ACG 250	T. Power	*fastigiata*	*fastigiata*	L
ACG 233	97-Vietname	*fastigiata*	*fastigiata*	L	ACG 251	96-Australia	*fastigiata*	*fastigiata*	L
ACG 234	98-Australia	*fastigiata*	*fastigiata*	S	ACG 252	Taianan	*fastigiata*	*fastigiata*	S
ACG 235	Florispan	*fastigiata*	*fastigiata*	L	ACG 253	H	*fastigiata*	*fastigiata*	S
ACG 236	1	*fastigiata*	*fastigiata*	L	ACG 254	Early rumir	*fastigiata*	*fastigiata*	L
ACG 237	18/38	*fastigiata*	*fastigiata*	L	ACG 255	Egret	*fastigiata*	*fastigiata*	S
ACG 238	Starr	*fastigiata*	*fastigiata*	L	ACG 256	DixilAnax	*fastigiata*	*fastigiata*	S

* *L* is resistant, *S* is susceptible, *N/A* is no amplification

10 × PCR buffer, 0.4 mM of dNTPs mix, 2.5 mM of $MgCl_2$, 0.3 µM each primer, 1 unit of *Taq* DNA polymerase (Fermentas Life Sciences, Burlington, Canada), 1 µl genomic DNA template and Milli-Q water to a final volume of 20 µl. The SSR marker (Forward primer: 5′ TACAGCATTGCCTTCTGGTG 3′; Reverse primer: 5′ GCACACCATGGCTCAGTT-ATT 3′), tightly linked to sclerotinia blight resistance gene (Chenault et al. 2009), amplification was performed in a programmable thermocycler (BIONEER, MyGenie^{TM}) under the following conditions: 94 °C for 2 min, 35 cycles of 94 °C for 45 s, annealing temperature 60 °C for 1 min, 72 °C for 90 s, and then a final extension of 10 min at 72 °C (Ferguson et al. 2004). PCR products were separated in 2–3 % agarose gels in 1× TBE buffer and visualized under UV light after staining with ethidium bromide. The expected bands were determined visually and recorded. Amplified products were also analyzed in the Fragment Analyzer^{TM} which is high resolution bio-imaging system (Advanced Analytical Technologies GmbH, Heidelberg, Germany). The DNF-900 Reagent Kit was used for qualitative analysis of DNA fragments ranging from 35 to 500 bp. The markers for 35 and 500 bp fragments were used for normalization, respectively. After analysis, virtual gel imaging was analyzed with the software PROSize 2.0 (Version 1.2.1.1) (Advanced Analytical Technologies, AMES, IA, USA). All reactions were performed twice. Amplified bands were scored as previously reported by Chenault et al. (2009). The authors identified four possible band amplifications (L, S, B, and b) using with the sclerotinia blight associated marker. If a genotype had only the 145 bp band, it was scored as L. When genotypes had predominant 145 and 100 bp bands, they were scored as B and b, respectively. Genotypes amplified only 100 bp band were given an S score.

Results and discussion

Molecular marker analysis was performed to screen sclerotinia blight resistance in groundnut collection. Two different band patterns (L and S) were observed in agarose gel (Fig. 1) and high bio-imaging system following to PCR amplification (Fig. 2). No genotypes had patterns B and b.

Chenault et al. (2009) stated that genotypes with 145 bp band were scored as "L" associated with sclerotinia blight resistance while genotypes carrying only 100 bp were given a score of "S" indicating

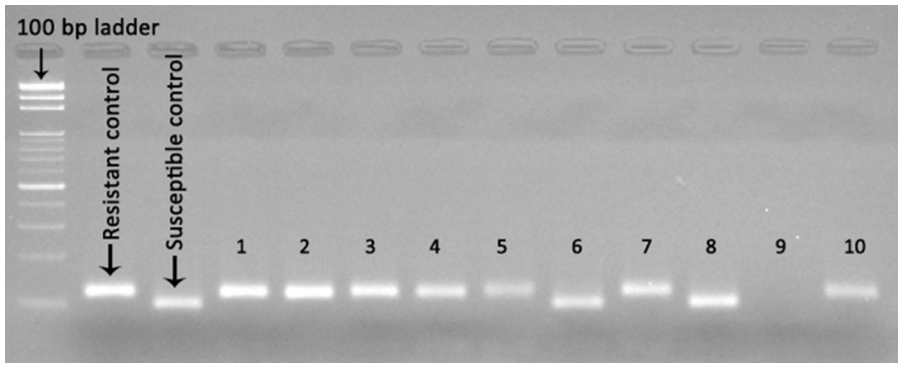

Fig. 1 Agarose gel showing the amplification products using sclerotinia blight associated marker. Resistant and susceptible controls are PI 482189 and PI 496448, respectively. The selected genotypes are numbered as follows: *lanes 1–10*, ACG 1; ACG 9; ACG 24; ACG 25; ACG 30; ACG 38; ACG 127; ACG 122; ACG 91; ACG 207

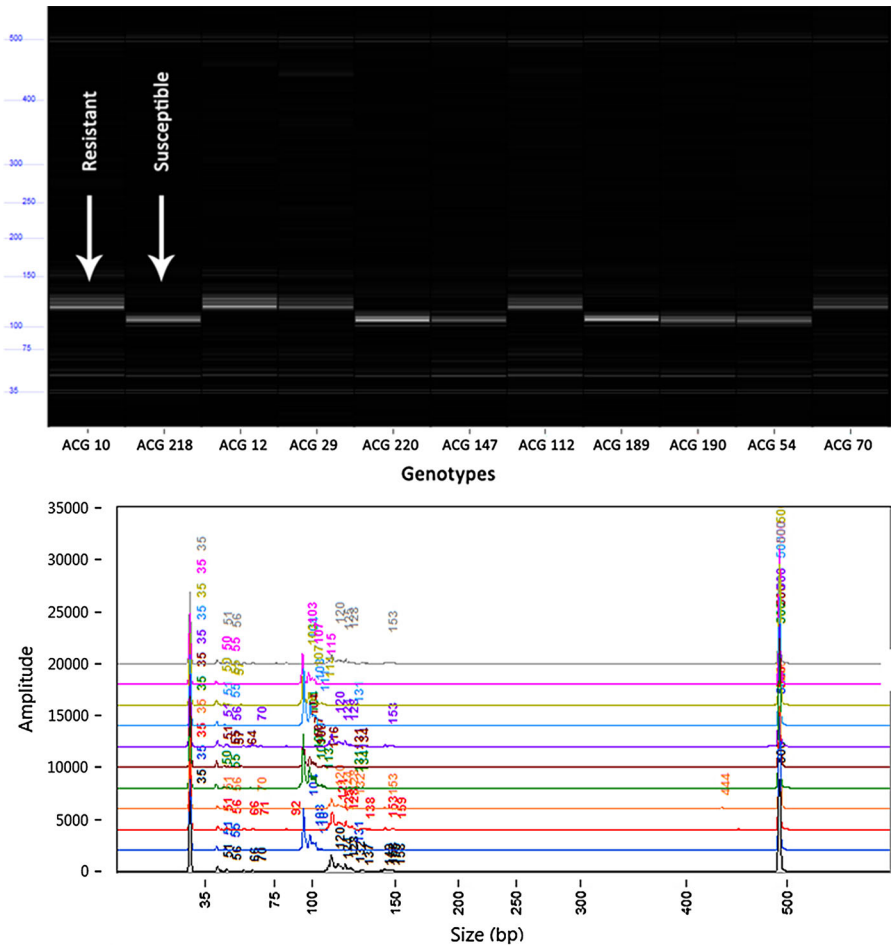

Fig. 2 Fragment Analyzer™ shows the gel picture and peak analysis graphic for the selected resistant/susceptible genotypes

susceptibility. In the present study, 142 genotypes showed the sclerotinia blight resistant fragment with 145 bp in the collection, while 100 bp band detected in 108 genotypes was associated with susceptibility (Table 1). Rest of six genotypes amplified no band following PCR amplification. The resistance allele in this study was present in higher frequency than susceptible resistance allele in the groundnut

Table 2 Botanical variety comparison of genotypes tested by the marker

Botanical variety	Marker score			
	L	S	B	b
hypogaea	27	101	–	–
fastigiata	54	5	–	–
vulgaris	56	3	–	–
peruviana	2	–	–	–
aequatoriana	1	–	–	–
hirsuta	1	–	–	–

collection. The present investigation therefore reports new sources of resistance to sclerotinia blight in groundnut. For a better comparison, in the U.S peanut mini core collection only 39 individuals from spanish, valencia, runner market types were classified as new potential sources of resistance (Chamberlin et al. 2010).

The cultivated groundnut has two subspecies, *A. hypogaea* ssp. *hypogaea* and *A. hypogaea* ssp. *fastigiata* (Gregory et al. 1980), and six botanical varieties *hypogaea*, *hirsuta*, *fastigiata*, *peruviana*, *aequuatoriana* and *vulgaris*. Moreover, the commercially grown cultivars are grouped into four market classes, the runner, virginia, spanish, and valencia market types (Krapovickas and Rigoni 1994). In the present study, the *vulgaris* types (39.4 %) were found more resistant to sclerotinia blight than those of *fastigiata* (38.0 %) and *hypogaea* (19.7 %) types (Table 2), with showing R banding pattern using the SSR marker among the resistant genotypes of the collection. This result highly compatible with the field study by Porter et al. (1975) who stated that spanish-type (variety *vulgaris*) groundnut has more resistance to sclerotinia blight than virginia types. Chenault et al. (2009) also identified high resistance in spanish types through molecular analysis.

This study included 186 groundnut mini core genotypes from ICRISAT. In the mini core collection, the R banding pattern related to the disease resistance is observed at high frequency in the variety *vulgaris* (48.6 %) and less distributed among the varieties *fastigiata* (31.3 %) and *hypoegaea* (17.3 %) among the resistant genotypes in the mini-core collection. Variety *peruviana* had only two genotypes in the collection and both of them consistent with resistance marker. The botanical variety *aequatoriana* was presented only one germplasm which was indicated resistance to sclerotinia blight disease after molecular analysis. The presence of sclerotinia blight resistance in the U.S. peanut mini core collection was also examined with molecular survey by Chamberlin et al. (2010). All spanish genotypes in that collection carrying S banding pattern which is less observed in the virginia (46 %) and runner market types (35 %). These findings confirm the usefulness of the sclerotinia blight resistant marker and effectiveness of MAS for groundnut breeding programs.

The wild species are good sources of sclerotinia blight resistance genes in groundnut (Tallury et al. 2014). However interspecific incompatibility and unfavorable linkage drag associated with resistance to diseases cause to limited success in transferring disease resistance (Murty and Jahnavi 1983). Molecular markers tightly linked to disease resistance loci may increase selection efficiency in interspecific derivatives (Mace et al. 2006). In this study, the disease resistant marker indicated that it could be directly used for marker-assisted breeding. However it is insufficient without yield traits. Groundnut is an industrial crop and comprehensive information or database on agronomic traits of all genotypes must be available for optimal commercial exploitation. The sclerotinia blight resistant sources identified from different botanical varieties in the present study therefore should be evaluated agronomically to provide better opportunities in developing high yielding resistant cultivars appropriate for different regions.

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Conflict of interest The authors declare that there are no conflicts of interest.

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