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ORIGINAL ARTICLE

Identification of expressed *R*-genes associated with leaf spot diseases in cultivated peanut

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Abstract

Peanut (*Arachis hypogaea* L.) is an important food and oilseed crop worldwide. Yield and quality can be significantly reduced by foliar fungal diseases, such as early and late leaf spot diseases. Acceptable levels of leaf spot resistance in cultivated peanut have been elusive due to environmental interactions and the proper combination of QTLs in any particular peanut genotype. Resistance gene analogs, as potential resistance (*R*)-genes, have unique roles in the recognition and activation of disease resistance responses. Novel *R*-genes can be identified by searches for conserved domains such as nucleotide binding site, leucine rich repeat, receptor like kinase, and receptor like protein from expressed genes or through genomic sequences. Expressed *R*-genes represent necessary plant signals in a disease response. The goals of this research are to identify expressed *R*-genes from cultivated peanuts that are naturally infected by early and late spot pathogens, compare these to the closest diploid progenitors, and evaluate specific gene expression in cultivated peanuts. Putative peanut *R*-genes (381) were available from a public database (NCBI). Primers were designed and PCR products were sequenced. A total of 214 sequences were produced which matched to proteins with the corresponding *R*-gene motifs. These *R*-genes were mapped to the genome sequences of *Arachis duranensis* and *Arachis ipaensis*, which are the closest diploid progenitors for tetraploid cultivated peanut, *A. hypogaea*. Identification and association of specific gene-expression will elucidate potential disease resistance mechanism in peanut and may facilitate the selection of breeding lines with high levels of leaf spot resistance.

Keywords Resistance gene analogs · RGAs · *R*-genes · Markers · Cultivated peanut · Disease resistance · Leaf spot · Genetic diversity

Introduction

In response to different disease pressures, plants have evolved intricate recognition and signal transduction systems to ward off pathogens. On the leaf surface, plants have different layers of waxes, hairs or trichomes, and a cell wall that act as physical barriers against non-adapted pathogens. At the cell surface, the presence of the pathogen is first recognized by receptor like kinases (RLKs) and receptor like proteins (RLPs) which function as pattern recognition receptors (PRRs) in interactions called pathogen/microbe-associated molecular patterns (PAMP/MAMP) to activate a pattern-triggered immunity (PTI) response [1]. Non-adaptive pathogens are usually stopped from entering plant cells at this point. Adapted pathogens can penetrate the cells to release pathogenic effector proteins and activate resistance (*R*) proteins of the host in a second line of defense, called effector triggered immunity (ETI) response [2]. In both PTI and ETI, plant activate an array of immune responses such

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as Ca^{2+} spike, reactive oxygen species (ROS) burst, MAP kinase (MAPK) activation, production of phytohormones, and modulation in transcriptional regulation [3].

Resistance (*R*) or effector–receptor gene candidates have been associated with plant disease resistance and have been identified in important crop plants based on conserved DNA motifs through genome sequencing and homologous gene cloning [4–6]. There are seven conserved motifs or domains: Toll/interleukin-1 receptor (TIR), leucine zipper (LZ), coiled–coiled (CC), nucleotide-binding site (NBS), leucine-rich repeat (LRR), transmembrane (TM), and serine-threonine kinase (STK) which can be broadly categorized into five main classes: TIR–NBS–LRR (TNL), CC–NBS–LRR (CNL), RLK, RLP, and other variations [2].

Peanut (*Arachis hypogaea* L.) is an important source of proteins, vitamins, and oil. It is grown in many parts of the world, with China and India as leading producers followed by Nigeria and USA [7]. Peanut is challenged by diseases, especially foliar diseases that have worldwide impact on yield and quality. Early leaf spot (ELS) caused by *Cercospora arachidicola* (Hori) and late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk. & M.A. Curtis) are important foliar fungal diseases that can cause complete defoliation and significantly reduce plant productivity. A combination of cultural practices such as crop rotation, proper management of residue by tillage practices [8], weather predictive models for disease outbreak [9, 10], and proper irrigation can minimize plant diseases. Application of fungicide can effectively control these diseases [11] but can be costly and maybe prohibited to subsistence peanut growing areas. Development of resistant peanut cultivars would be a sustainable solution for many parts of the world.

Because of the polyploidy nature of the cultivated peanut and the low DNA marker polymorphisms, progress in the application of marker-assisted plant breeding has been difficult. A large number (> 10,000) of simple sequence repeat (SSR) potential markers are available, but < 7% are polymorphic among cultivated peanuts [12]. Validated marker-trait associations for nematode resistance and high oleic chemistry have been applicable in breeding programs [13, 14]. Recent research utilizing a recombinant inbred line (RIL) population that segregated for quantitative field resistance to LLS identified several quantitative trait loci (QTLs) [15]. Even with the discovery of a few candidate gene markers, application of marker-trait association continues to be a challenge since field performance evaluation, or phenotyping, can be significantly variable based on year, location, or environmental differences. Furthermore, defense responses and disease resistance (*R*)-gene activation have a fitness cost which can reduce plant growth and production [16]. In nature, plants select the 'perfect' combination of genes and coordinate gene-regulatory patterns necessary to ensure survival and productivity [17].

Recently, significant progress has been made in peanut genomics research which culminated in the sequencing of the two closest diploid peanut progenitors, *Arachis duranensis* and *Arachis ipaensis* [18], and the cultivated allotetraploid peanut (*A. hypogaea*) is now available through PeanutBase.org. Through a concerted genomics effort, there are currently 281,451 ESTs and a composite of 50,777 transcriptome shotgun assemblies (TSAs) archived on NCBI database. A first generation (58K) and a second generation (48K) single nucleotide polymorphism (SNP) chips have been utilized in different gene-expression experiments [19, 20]. These resources, as well as other bioinformatic projects in peanuts, provide a tremendous platform to identify functional genes that can lead to the development of disease resistant peanut varieties and was utilized to identify resistance gene analogs (RGAs), as resistance (*R*)-genes, associated with ELS and LLS. In peanuts, 78 RGAs were identified from peanut cultivar 'Tahu' and four diploid species [21] and later Yuksel et al. [22] identified 234 RGAs from cultivars 'Florunner' and 'UF-439-16-1003-2' from genomic DNA. Liu et al. [23] integrated previously identified RGAs with new EST sequences available at the time and derived 385 putative RGAs (156 contigs and 229 singletons).

The goals of this research are to identify and clone expressed *R*-gene candidates in peanut plants challenged with ELS and LLS pathogens and to associate these sequences with molecular pathways that may be used as disease resistant gene markers for peanut variety development. Gene-expression profiling of transcribed *R*-gene candidates in peanuts challenged with diseases provide a more comprehensive picture of disease resistance gene-regulation network and facilitate future peanut breeding.

Materials and methods

Identification of *R*-genes through database search

RGA sequences were utilized from different groups [21–23]. All subsequent sequence nomenclatures are loosely assigned RGAs, or *R*-genes, to include all five major classes [2]. Sequence analyses were performed using Sequencher DNA analysis software (Gene Codes, Ann Arbor, MI, USA). Unique sequences with potential open reading frame (ORF) and with low E-value in BLASTx search (NCBI) results were selected for analysis. Sequences were searched against all *Arachis* EST and TSA NCBI databases (Online Resource 1). Sequences of each EST and TSA were downloaded and re-assembled to verify uniformity of each alignment and to obtain longer ORFs. Newly assembled sequences were evaluated to ensure the presence of an ORF and returned a significant BLASTx and HMMER (EMBL-EBI) matches to proteins with *R*-gene motifs [2]. Sequences that did not

have ORFs and did not match to potential *R*-genes were not evaluated further.

Peanut genotypes and plant treatment

Two peanut varieties (Flavorrunner (FR) 458 and Georgia (Ga) 12Y), a breeding line (Exp27-1516), and a PI 268868 were evaluated. FR458, released in 1996, is a runner-type peanut that is highly susceptible to most peanut diseases and is utilized as a susceptible check to tomato spotted wilt (TSW), caused by *Tomato spotted wilt virus* [24]. Ga12Y, released in 2012, is also a runner-type peanut with resistance to TSWV and white mold or stem rot (caused by *Sclerotium rolfsii Sacc.*) [25]. Exp27-1516, a runner-type with medium resistance to ELS and LLS resistance and highly resistant to TSWV, was provided by Dr. Charles Chen (Auburn University) through a USDA/Auburn joint breeding program. PI 268868, a Virginia-type peanut with observed field resistance to ELS and LLS, was kindly provided by the USDA peanut germplasm repository in Griffin, GA. Seeds were planted at the rate of 6 seeds per 1 m row, with 6 row replicates randomly distributed in a 5.5 × 12 m plastic house with screens on the sides for open air. Best agricultural plant treatment was utilized and no fungicides were applied throughout the growing season.

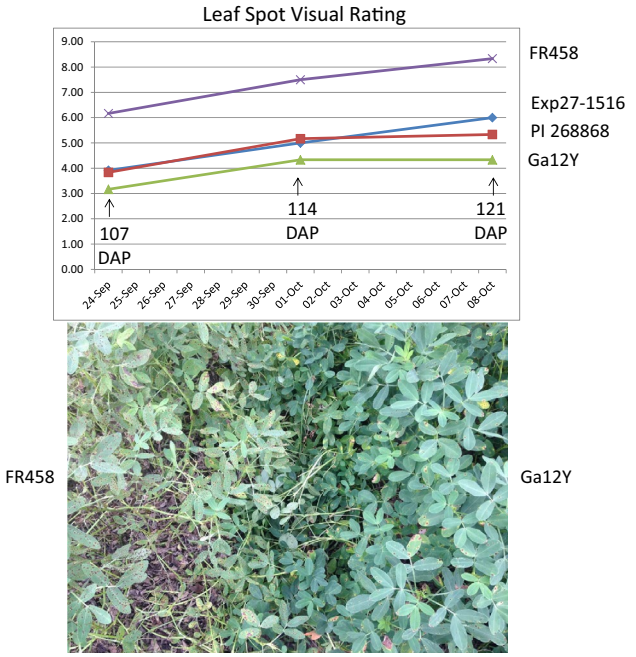


Fig. 1 Leaf spot progressive disease ratings, comparing four peanut genotypes near the end of the peanut growing season (top). These evaluations were based on a Florida scale from 1 to 10 with 1 = no symptom and 10 = complete defoliation. Picture of FR458 (susceptible) and Ga12Y (tolerant) to late-season leaf spot disease at 107 DAP (bottom)

Leaf spot disease evaluation and sample collection

Visual assessment of leaf spot disease severity was based on a Florida 1–10 scale where 1 represents no disease or visual symptoms and 10 is complete leaf defoliation [26]. Leaf spot symptoms were assessed at three dates near the end of the growing season (107, 114 and 121 days after planting, DAP) (Fig. 1). Leaf samples were collected at 121 DAP (3rd assessment), following the disease rating, for RNA analysis. This developmental stage represents late-season leaf spot infection, culminating to severe plant disease response and correlates to significant yield losses without fungicide applications. Fully expanded leaves were collected from a prominent stem from four randomly selected plants in 1 m linear row. Round punches (2 cm) of each leaf from four plants were pooled, placed into a 2 mL tube, frozen and stored at − 80 °C until processed.

RNA extraction, cDNA synthesis and PCR product sequencing

Total RNAs from fresh-frozen peanut leaves were extracted utilizing TRIzol Reagent (Ambion, Austin, TX, USA) according to manufacturer's instruction. RNA was quantified using Nanodrop 2000 spectrophotometer (ThermoFisher Sci. Waltham, MA, USA) and quality was determined based on agarose gel electrophoresis analysis. RNA was DNase-treated with Turbo DNA-free (Ambion) prior to cDNA synthesis. 1 µg total RNA was used as template and cDNAs were produced according to Dang et al. [27]. cDNAs were diluted 1:10 with sterile water and used as template in standard PCR reaction. Primers were designed using Clone Manager (Sci-Ed Software, Denver, CO, USA) to obtain the largest ORF sequence possible for each predicted RGA (Online Resource 2). The 20 µL PCR reaction consisted of 3 µL of diluted cDNAs, 10 µL GoTaq Green Master mix (Promega, Madison, WI, USA) and 0.4 µM of each primer, with cycling conditions of 2 min at 94 °C to completely denature cDNAs, followed by 40 cycles of 20 s at 94 °C, 20 s at 55 °C and 50 s at 72 °C, and a final cycle 10 min at 72 °C to produce complete PCR products. PCR products were resolved on 1% TAE gel-electrophoresis, single bands at the predicted molecular weight were isolated and purified utilizing QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA), and 80 ng of purified-PCR products were sent for dideoxy-chain termination method sequencing (Eurofins MWG Operon, Louisville, KY, USA) with the forward or reverse specific primer.

Cloning of PCR products, plasmid isolation and sequencing

Gel purified PCR products (50 ng) were cloned using StrataClone PCR Cloning Kit (Stratagene, San Diego, CA, USA). Single bacteria-colonies were selected and grown overnight at 37 °C with shaking with ampicillin antibiotic selection. Plasmids were extracted using QIAprep Spin Miniprep kit (Qiagen) and purified plasmids (300 ng) were sequenced (Eurofins) with T3 or T7 promoter sequencing primers.

Quantitative (q) RT-PCR

Diluted cDNAs were used as template in real-time fluorescence qRT-PCR with specific gene primers (Online Resource 3). Data was generated on QuantStudio7 Flex real-time PCR system (ThermoFisher Sci. Waltham, MA, USA) utilizing relative quantitation method as described by manufacturer. The 20 µL reaction consisted of 3 µL of diluted cDNAs, 10 µL PowerUp SYBR green master mix (ThermoFisher Sci.) and 0.4 mM of each primer, with PCR cycling conditions of 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 58 °C, and a dissociation curve analysis cycle of 15 s at 95 °C, 20 s at 58 °C and 15 s at 95 °C. The threshold cycle (Ct) was automatically calculated by QuantStudio Real-Time PCR software (ThermoFisher Sci.) and relative expression was calculated based on $2^{-\Delta\Delta Ct}$ described by Livak and Schmittgen [28]. All samples were first normalized to *Actin* (EZ723877) as an internal control then transformed data were normalized with FR458 $2^{-\Delta\Delta Ct}$ values and compared with the other three peanut genotypes to determine relative fold changes in gene-expression.

Results

Identification of potential *R*-genes

From over 400 initial *R*-gene candidate sequence targets, 381 were observed to have ORFs and matched BLASTx to proteins with *R*-gene motifs. These sequence sizes ranged from 404 to 3582 bp. Primers were designed to cover a large segment of predicted ORFs. Reverse-transcribed PCR analysis, utilizing RNA from leaf spot infected leaves, identified 241 primer-pairs that resulted in PCR products on agarose gel-electrophoresis. PCR products were purified and sequenced, resulting in a total of 214 RGA transcripts that produced ORFs and matched to an *R*-gene motif in BLASTx and HMMER searches (Table 1).

Conservation of *R*-genes in coding region

SNPs were observed and reported for each *R*-gene (Table 2). From the 214 candidate RGAs, 172 produced observable PCR bands in four peanut genotypes and these products were cloned and sequenced. When sequencing results were compared, 86 RGAs had 0 SNP and 86 had between 1 and 16 SNPs in their respected DNA sizes (232–1776 bp). From the same set, 107 were identified to be single copy genes when electronically mapped to *A. duranensis* or *A. ipaensis* genomes, 64 had 2–5 allelic variants, and 2 had 7–10 variants.

Discovery of insertions/deletions (indels)

From the 214 RGAs identified, four indels were discovered through PCR product cloning. RGA 14a has a 1074 bp length with a 6 bp indel. Blastx search matched to a serine/threonine kinase HT1-like protein. RGA108, 348 bp in length containing a 9 bp indel, codes for a TMV resistance N-like protein. RGA188, 378 bp in length with a 3 bp indel, codes for a receptor-like protein 12. RGA322a, 1369 bp in length with a 3 bp indel, codes for a receptor-like protein kinase 5. These RGAs are all inframe indels that has potential add function to native transcripts.

Mapping *R*-genes to peanut diploid genomes

These sequences were searched using Blastn algorithm in NCBI database selecting *Arachis* as search organism. Verified sequencing transcripts were electronically mapped to *A. duranensis* or *A. ipaensis* genomes using Blastn algorithm utilizing NCBI nucleotide database (Table 3). Eighteen RGAs were mapped to *A. duranensis* (chromosome A01) and only 14 of the same RGAs mapped to *A. ipaensis* (B01), with 4 RGAs mapped to different *A. ipaensis* chromosomes. Thirteen RGAs mapped to *A. duranensis* (A02) and same RGAs were also mapped to *A. ipaensis* (B02), with an additional RGA108 mapped only to *A. ipaensis*. On chromosome 3, 34 RGAs were mapped to *A. duranensis* (A03) while only 32 mapped to *A. ipaensis* (B03). Twelve RGAs mapped to both *A. duranensis* (A04) and *A. ipaensis* (B04), with RGA 34 mapped only to *A. duranensis* (A04). RGAs 123, 265 and 293 mapped only to *A. ipaensis* (B04). On *A. duranensis* (A05) and *A. ipaensis* genomes (B05), 32 RGAs were mapped to both diploid chromosomes with an additional RGA 99 only present on *A. duranensis* genome (A05). Ten RGAs were mapped to both *A. duranensis* (A06) and *A. ipaensis* (B06) chromosomes, with an additional two RGAs 202 and 216 present on *A. ipaensis* genome (B06). Out of the 25 RGAs mapped to either *A. duranensis* (A07) or *A. ipaensis* (B07) chromosomes, only 11 were present on both diploid chromosomes. RGAs 91b, 170, 198, 202, 341

Table 1 Identification of conserved *R*-gene motifs by HMMER and BLASTX searches and the associated protein functions

ID	Domain	Class	Blastp description	qRT-PCR
RGA002	TNL	NBS	TMV resistance protein N-like	
RGA003	LRR_RI	RLP	Plant intracellular Ras-group-related LRR protein 7	*Up*
RGA004	LRR_STKc	RLK	Receptor protein kinase TMK1	
RGA007	STKc	Other	Serine/threonine-protein kinase At5g01020	
RGA009	LRR_TM	RLP	DNA-damage-repair/toleration protein DRT100-like	
RGA012	LRR_STKc	RLK	Receptor-like protein kinase At1g35710	
RGA013	CNL	NBS	Disease resistance protein RPP13	
RGA014a	STKc_MAP3K	Other	Mitogen-activated protein kinase kinase kinase	
RGA016a	STKc	Other	Pto-interacting protein	
RGA017	LRR_STKc	RLK	Somatic embryogenesis receptor kinase	Down
RGA020	LRR_STKc	RLK	Receptor-like protein kinase At5g47070	*Up*
RGA021	LRR_STKc	RLK	Receptor-like serine/threonine-protein kinase At1g17230	
RGA023	STKc	Other	Serine/threonine-protein kinase PBS1	**Down**
RGA025	STKc	Other	Serine/threonine-protein kinase CDL1-like	
RGA027	LRR_STKc	RLK	Receptor kinase At5g58300	
RGA028	STKc	Other	Serine/threonine-protein kinase Cx32, chloroplastic	*Up*
RGA031	STKc	Other	Serine/threonine-protein kinase CDL1-like	
RGA031a	STKc	Other	Serine/threonine-protein kinase CDL1-like	
RGA033	LRR_STKc	RLK	receptor-like protein kinase PEPR1	
RGA034	LRR_STKc	RLK	Serine/threonine-protein kinase BAM3	
RGA035	STKc	Other	Serine/threonine-protein kinase	*Up*
RGA036	Mlo	Other	MLO-like protein	
RGA037	STKc	Other	Serine/threonine-protein kinase At1g01540	
RGA040b	STKc	Other	Cysteine-rich receptor-like protein kinase	Down
RGA041	LRR_STKc	RLK	Uncharacterized protein	
RGA042	LRR_STKc	RLK	Proline-rich receptor-like protein kinase PERK1	Up
RGA044	TIR	NBS	Toll/interleukin-1 receptor-like protein	
RGA047	TNL	NBS	TMV resistance protein N-like	
RGA049	LRR_TM	RLP	Polygalacturonase inhibitor-like	
RGA051	LRR_TM	RLP	DNA-damage-repair/toleration protein DRT100-like	**Down**
RGA052	LRR_STKc	RLK	Receptor-like serine/threonine-protein kinase At1g34110	
RGA054	LRR_STKc	RLK	Receptor-like serine/threonine-protein kinase FEI 1	*Up*
RGA055	STKc	Other	Serine/threonine-protein kinase Cx32, chloroplastic	*Up*
RGA057	LRR_STKc	RLK	Receptor-like protein kinase At5g47070	*Up*
RGA058	NL	NBS	TMV resistance protein N-like	
RGA059	STKc	Other	Serine/threonine-protein kinase At5g01020	
RGA060	TIR	NBS	TMV resistance protein N	**Down**
RGA061	TIR	NBS	TMV resistance protein N	
RGA062	LRR_STKc	RLK	Receptor-like kinase TMK4	*Up*
RGA065	LRR_STKc	RLK	Proline-rich receptor-like protein kinase PERK9	**Down**
RGA068	TIR	NBS	TMV resistance protein N-like	Up
RGA069	LRR_TM	RLP	Piriformospora indica-insensitive protein 2-like	
RGA070	STKc	Other	Rust resistance kinase Lr10-like	
RGA073	STKc	Other	Protein kinase 2B, chloroplastic-like	Mix
RGA073a	STKc	Other	Protein kinase 2B, chloroplastic-like	
RGA075	LRR_STKc	RLK	Receptor-like protein kinase At4g00960	
RGA078	STKc_MAPKK	Other	Mitogen-activated protein kinase kinase	Mix
RGA079	LRR_STKc	RLK	Receptor-like protein kinase HERK 1	
RGA082	TIR	NBS	TMV resistance protein N	*Up*
RGA084	LRR_STKc	RLK	Serine/threonine-protein kinase FLS2	

Table 1 (continued)

ID	Domain	Class	Blastp description	qRT-PCR
RGA085	TIR	NBS	TMV resistance protein N-like	
RGA086	TIR	NBS	TMV resistance protein N-like	Mix
RGA087a	LRR_STKc	RLK	Receptor-like serine/threonine-protein kinase At4g34500	
RGA091b	STKc	Other	PTI1-like tyrosine-protein kinase At3g15890	**Down**
RGA092	STKc_MAP3K	Other	Mitogen-activated protein kinase kinase kinase	*Up*
RGA097	LRR_STKc	RLK	Receptor-like protein kinase FERONIA	
RGA098	Hs1pro	Other	Nematode resistance protein-like HSPRO2	Down
RGA099	STKc	Other	Protein kinase 2A, chloroplastic-like	Down
RGA099a	STKc	Other	Protein kinase 2B, chloroplastic-like	
RGA100	LRR_TM	RLP	Receptor-like protein 12	*Up*
RGA101a	STKc	Other	STRUBBELIG-RECEPTOR FAMILY 6	*Up*
RGA102a	STKc	Other	Serine/threonine-protein kinase PBS1	**Down**
RGA103	LRR_STKc	RLK	Serine/threonine-protein kinase BAM1	
RGA105	LRR_RI	RLP	Polygalacturonase inhibitor 2-like	
RGA106	LRR_STKc	RLK	Serine/threonine-protein kinase At4g36180	*Up*
RGA107	LRR_STKc	RLK	Phytosulfokine receptor 2	
RGA108	TIR	NBS	TMV resistance protein N-like	**Down**
RGA110	LRR_STKc	RLK	Receptor-like protein kinase FERONIA	
RGA113a	STKc	Other	Serine/threonine-protein kinase CDL1	Down
RGA116	LRR_RI	RLP	Polygalacturonase inhibitor 2-like	**Down**
RGA121a	LRR_STKc	RLK	Receptor-like kinase TMK4	Up
RGA123a	LRR_TM	RLP	Uncharacterized receptor-like protein	**Down**
RGA124	LRR_TM	RLP	DNA-damage-repair/toleration protein DRT100-like	
RGA125	TNL	NBS	TMV resistance protein N-like	
RGA126	TNL	NBS	TMV resistance protein N-like	
RGA127	TIR	NBS	Uncharacterized protein	**Down**
RGA129	LRR_STKc	RLK	Somatic embryogenesis receptor kinase 2-like	
RGA130	LRR_TM	RLP	Receptor-like protein 12	
RGA131	C-CAP	Other	Adenylyl cyclase-associated protein	
RGA132	STKc	Other	Serine/threonine-protein kinase CDL1-like	
RGA139	TNL	NBS	Disease resistance protein At3g14460	
RGA140	STKc	Other	PTI1-like tyrosine-protein kinase 3	*Up*
RGA141	STKc	Other	Uncharacterized protein	
RGA144	STKc	Other	STRUBBELIG-RECEPTOR FAMILY 7-like	Up
RGA147b	STKc_MAP3K	Other	Mitogen-activated protein kinase kinase kinase	**Down**
RGA148	STKc	Other	Serine/threonine-protein kinase CDL1	Down
RGA151	STKc	Other	Serine/threonine-protein kinase-like protein At3g51990	
RGA152	STKc	Other	Calmodulin-binding receptor-like cytoplasmic kinase 2	Mix
RGA153b	STKc	Other	Serine/threonine-protein kinase SD1-8	**Down**
RGA154a	STKc	Other	Uncharacterized protein	**Down**
RGA157	LRR_TM	RLP	Disease resistance protein RGA1	
RGA161	STKc	Other	Serine/threonine-protein kinase BIK1-like	Mix
RGA162	STKc	Other	Protein kinase 2B, chloroplastic-like	*Up*
RGA163	STKc	Other	Chitin elicitor receptor kinase 1-like	
RGA165	STKc	Other	Uncharacterized protein	
RGA166	LRR_STKc	RLK	Serine/threonine-protein kinase RPK2	**Down**
RGA170	LRR_STKc	RLK	Pollen receptor-like kinase 4	
RGA171	LRR_STKc	RLK	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase	
RGA172	STKc_MAP3K	Other	Mitogen-activated protein kinase kinase kinase	Mix
RGA177	NL	NBS	Disease resistance protein At4g27220	

Table 1 (continued)

ID	Domain	Class	Blastp description	qRT-PCR
RGA178	Lectin_STKc	Other	L-type lectin-domain containing receptor kinase	
RGA179	STKc_MAP3K	Other	Serine/threonine-protein kinase CTR1-like	Mix
RGA181	Lectin-STKc	Other	G-type lectin S-receptor-like serine/threonine-protein kinase	**Down**
RGA188	LRR_STKc	RLK	Receptor-like protein kinase	
RGA189	STKc	Other	Wall-associated receptor kinase-like 20	
RGA191	STKc	Other	Receptor-like protein kinase HERK 1	
RGA192	Glyco_18	Other	Cysteine-rich receptor-like protein kinase 10	
RGA197	STKc	Other	Protein kinase 2B, chloroplastic-like	
RGA198	STKc	Other	Uncharacterized protein	**Down**
RGA199	STKc	Other	Receptor-like protein kinase At5g15080	*Up*
RGA201	LRR_STKc	RLK	Serine/threonine-protein kinase GSO2	*Up*
RGA202	STKc	Other	Serine/threonine-protein kinase NAK	**Down**
RGA204	Lectin-STKc	Other	L-type lectin-domain containing receptor kinase	
RGA206	LRR_TM	RLP	Disease resistance protein At5g66900	*Up*
RGA207	LRR_STKc	RLK	Serine/threonine-protein kinase RPK2	Up
RGA208	TIR	NBS	TMV resistance protein N-like	**Down**
RGA210a	LRR_TM	RLP	Disease resistance protein RML1A-like	
RGA211	NL	NBS	Disease resistance protein RGA4	
RGA212	STKc	Other	Uncharacterized protein	
RGA213	LRR_STKc	RLK	Serine/threonine-protein kinase At1g17230	Mix
RGA215	STKc	Other	Wall-associated receptor kinase-like 14	**Down**
RGA216	LRR_STKc	RLK	LRR receptor-like kinase	
RGA218	LRR_TM	RLP	Receptor-like protein 12	
RGA222	STKc	Other	Pto-interacting protein 1-like	**Down**
RGA223	Lectin_STKc	Other	G-type lectin S-receptor-like serine/threonine-protein kinase	
RGA226	Lectin-STKc	Other	L-type lectin-domain containing receptor kinase	**Down**
RGA229	LRR_TM	RLP	Extensin-like protein 4	
RGA233	STKc	Other	Protein LYK5	
RGA234	LRR_STKc	RLK	Receptor-like protein kinase HAIKU2	
RGA235	LRR_TM	RLP	BRASSINOSTEROID INSENSITIVE 1-like	*Up*
RGA236	LRR_STKc	RLK	LRR receptor-like kinase	
RGA237	STKc_MAP3K	Other	Mitogen-activated protein kinase kinase kinase	**Down**
RGA238	STKc	Other	Uncharacterized protein	**Down**
RGA240	TIR	NBS	Disease resistance RPP13-like protein	Up
RGA245a	LRR_STKc	RLK	Receptor-like protein kinase At5g24010	**Down**
RGA245b	LRR_STKc	RLK	Receptor-like protein kinase FERONIA	
RGA246	LRR_STKc	RLK	Receptor-like protein kinase At2g33170	**Down**
RGA249a	LRR_STKc	RLK	Receptor-like protein kinase At5g47070	**Down**
RGA249b	LRR_STKc	RLK	Receptor-like protein kinase At5g47070	Down
RGA250	LRR_STKc	RLK	Receptor-like protein kinase At1g35710	*Up*
RGA251	LRR_STKc	RLK	Serine/threonine-protein kinase RPK2	**Down**
RGA252	LRR_STKc	RLK	Wall-associated receptor kinase-like 20	
RGA253	LRR_TM	RLP	TMV resistance protein N-like	
RGA253a	LRR_TM	RLP	disease resistance protein RPS6-like	**Down**
RGA255	LRR_STKc	RLK	Receptor-like protein kinase HAIKU2	Mix
RGA257	LRR_STKc	RLK	Receptor-like protein kinase PXL2	
RGA259	STKc	Other	Wall-associated receptor kinase-like 14	
RGA260	STKc	Other	Uncharacterized protein	Up
RGA261a	LRR_STKc	RLK	LRR receptor-like serine/threonine-protein kinase	
RGA265	TIR	NBS	TMV resistance protein N-like	*Up*

Table 1 (continued)

ID	Domain	Class	Blastp description	qRT-PCR
RGA266	TIR	NBS	Uncharacterized protein	
RGA268a	LRR_TM	RLP	Disease resistance protein At3g14460	
RGA269	ATPase	Other	Plasma membrane ATPase 1-like	
RGA270	TNL	NBS	TMV resistance protein N-like	*Up*
RGA275a	STKc	Other	Serine/threonine-protein kinase	
RGA276	TNL	NBS	TMV resistance protein N-like	
RGA278	Lectin-STKc	Other	L-type lectin-domain containing receptor kinase	
RGA278a	Lectin-STKc	Other	L-type lectin-domain containing receptor kinase	
RGA286	LRR_STKc	RLK	Receptor-like protein kinase At5g15080	*Up*
RGA288	Lectin-STKc	Other	L-type lectin-domain containing receptor kinase	Mix
RGA289	LRR_STKc	RLK	Serine/threonine/tyrosine-protein kinase SOBIR1	
RGA290	LRR_TM	RLP	Disease resistance RPP13-like	
RGA292	LRR_TM	RLP	Receptor-like protein 12	
RGA293	STKc	Other	Wall-associated receptor kinase-like	
RGA296	TIR	NBS	TMV resistance protein N-like	
RGA297b	STKc	Other	STRUBBELIG-RECEPTOR FAMILY 3-like	
RGA298	NL	NBS	TMV resistance protein N-like	
RGA300	LRR_TM	RLP	Extensin-like protein 6	
RGA301	LRR_STKc	RLK	Receptor protein kinase TMK1-like	
RGA301a	LRR_STKc	RLK	Receptor protein kinase TMK1-like	
RGA303	STKc	Other	Phytosulfokine receptor 1	
RGA304	LRR_STKc	RLK	Receptor-like serine/threonine-protein kinase BAM1	*Up*
RGA307	LRR_STKc	RLK	Uncharacterized protein	**Down**
RGA310	LRR_STKc	RLK	LEAF RUST DISEASE-RESISTANCE RECEPTOR PROT KINASE	
RGA312	LRR_STKc	RLK	Receptor-like serine/threonine-protein kinase At4g26540	
RGA313	TNL	NBS	TMV resistance protein N-like isoform	
RGA314	LRR_STKc	RLK	Receptor-like protein kinase HSL1	*Up*
RGA315	TIR	NBS	TMV resistance protein N-like	*Up*
RGA318	NL	NBS	TMV resistance protein N-like	**Down**
RGA319	LRR_STKc	RLK	Receptor-like serine/threonine-protein kinase IRK	
RGA321a	LRR_STKc	RLK	Receptor-like serine/threonine-protein kinase BAM1	*Up*
RGA322	LRR_STKc	RLK	Receptor-like protein kinase HSL1	Mix
RGA322a	LRR_STKc	RLK	Receptor-like protein kinase 5	
RGA327a	STKc	Other	PTI1-like tyrosine-protein kinase	
RGA330	LRR_STKc	RLK	BRASSINOSTEROID INSENSITIVE 1-like	
RGA331	LRR_STKc	RLK	LRR receptor-like serine/threonine-protein kinase	
RGA336	STKc	Other	Mitogen-activated protein kinase homolog MMK2-like	**Down**
RGA337	STKc	Other	Serine/threonine-protein kinase At1g01540	
RGA338	STKc	Other	Protein kinase APK1B, chloroplastic-like	**Down**
RGA340	TIR	NBS	TMV resistance protein N-like	*Up*
RGA341	STKc	Other	Uncharacterized protein	*Up*
RGA342	LRR_STKc	RLK	Receptor-like protein kinase At5g56460	
RGA343	NL	NBS	Disease resistance protein RPM1-like	
RGA344	LRR_TM	RLP	Receptor-like protein 12	
RGA345	LRR_TM	RLP	Receptor-like protein 12	
RGA347	LRR_STKc	RLK	Receptor protein kinase MSP1-like	
RGA348	LRR_STKc	RLK	Receptor-like protein kinase At5g48380	*Up*
RGA349	LRR_STKc	RLK	Receptor protein kinase EMS1	
RGA352	STKc	Other	Serine/threonine-protein kinase BRI1-like 2	
RGA354	LRR_STKc	RLK	Somatic embryogenesis receptor kinase	

Table 1 (continued)

ID	Domain	Class	Blastp description	qRT-PCR
RGA355	STKc	Other	Calmodulin-binding receptor-like cytoplasmic kinase	*Up*
RGA359	STKc_Ubox	Other	U-box domain-containing protein	*Up*
RGA360	STKc	Other	Receptor-like protein kinase At5g18500	
RGA362	STKc	Other	BRASSINOSTEROID INSENSITIVE 1-associated recept kinase	
RGA364	STKc	Other	Receptor-like protein kinase At2g42960	
RGA365	STKc_Ubox	Other	U-box domain-containing protein	Mix
RGA366	LRR_STKc	RLK	Receptor-like serine/threonine-protein kinase At1g74360	
RGA369	LRR_STKc	RLK	Receptor-like serine/threonine-protein kinase At1g74360	
RGA370	STKc	Other	Glycerophosphodiester phosphodiesterase protein kinase	
RGA374	LRR_STKc	RLK	Receptor protein kinase MSP1-like	
RGA375	Lectin-STKc	Other	G-type lectin S-receptor protein kinase	
RGA377	STKc_MAP3K	Other	Mitogen-activated protein kinase kinase kinase	
RGA379	LRR_STKc	RLK	Receptor-like serine/threonine-protein kinase BIR2	
RGA384	LRR_STKc	RLK	Receptor-like serine/threonine-protein kinase At1g12460	

Real time qPCR results showed relative up- or down-regulations of *R*-genes. Positively correlated genes (labeled in italic) and negatively correlated genes (labeled in bold) are potential gene-expression markers for leaf spot resistance in peanut

and 374 were mapped only to *A. duranensis* (A07), while RGAs 27, 31, 36, 146, 148, 215, 314 and 369 mapped to *A. ipaensis* (B07) but were present on different *A. duranensis* chromosomes. Out of the 26 mapped to both *A. duranensis* (A08) and *A. ipaensis* (B08) chromosomes, 14 RGAs were represented in both. RGAs 3, 31, 36, 215, 314, 315, 366 and 369 were present on *A. duranensis* genome (A08) while same RGAs were on different *A. ipaensis* chromosomes. Fourteen RGAs were present on both *A. duranensis* (A09) and *A. ipaensis* (B09) chromosomes, while RGAs 91b and 170 were on *A. ipaensis* (B09) but on different *A. duranensis* chromosomes. RGA208 was only mapped to *A. duranensis* (A09) and not present on any *A. ipaensis* chromosomes. Out of the 25 RGAs represented on *A. duranensis* (A10) and *A. ipaensis* (B10) chromosomes, 16 RGAs were mapped on both diploid chromosomes. RGAs 27 and 148 were mapped to *A. duranensis* (A10) but on different *A. ipaensis* chromosomes. RGAs 3, 28, 54, 141, 366 and 374 were present on *A. ipaensis* (B10) but on different *A. duranensis* chromosomes. RGA165 was present on *A. ipaensis* genome (B10) but absent from any *A. duranensis* chromosomes.

Relative gene-expression and correlation to leaf spot resistance

Real time qPCR primers were designed and tested for efficiency (Online Resource 3). From the 89 RGAs that were evaluated for qRT-PCR, 39 were up-regulated and 38 were down-regulated (Fig. 2a, b). The remaining 12 were both up- and down-regulated (mix) among the 4 peanut genotypes tested. From the 39 up-regulated genes, 28 were identified as RLKs, 4 were RLPs, and 7 were TNLs. From the 38 down-regulated genes, 28 were RLKs, 5 were RLPs, and 5 were TNLs. From the remaining 12 genes, 10 were RLKs, 1 was an RLP, and 1 was a TNL. From the all 13 TNLs, 12 were associated with TMV resistance protein N-like and 1 code for a disease resistance RPPP13-like protein. When leaf spot susceptible peanut variety (FR458) was compared to the other 3 (more tolerant) peanut genotypes, 32 *R*-genes were positively correlated (labeled in italic) and 32 *R*-genes were negatively correlated (labeled in bold) with gene-expression levels (Table 1). These 64 candidate genes are potential gene-expression markers that can be utilized to select leaf spot resistance in peanut breeding programs.

Discussions

Plants are challenged with adverse biotic and abiotic pressures which require constant monitoring and modulating protective mechanisms, yet maintaining high productivity. For example, plants grown in high disease environments would invest more energy to maintain a "ready" state or be on a constant induction of disease responsive genes. RGAs, as *R*-genes, are essential in the plant immune system and are not well characterized in peanuts. From that aspect, a systematic approach was utilized to identify and sequence expressed *R*-genes in response to ELS and LLS pathogens.

Lately, there has been progress on the introgression and the identification of QTLs associated with ELS and LLS resistance [15, 29–32]. Because of a high number of QTLs and strong G × E interactions, predicting consistent disease resistance traits across different peanut genotypes is difficult. Identification of expressed *R*-genes in cultivated

Table 2 RGAs associated with the numbers of SNPs and the predicted allelic variants observed in *A. duranensis* (D) and *A. ipaensis* (I) diploid genomes

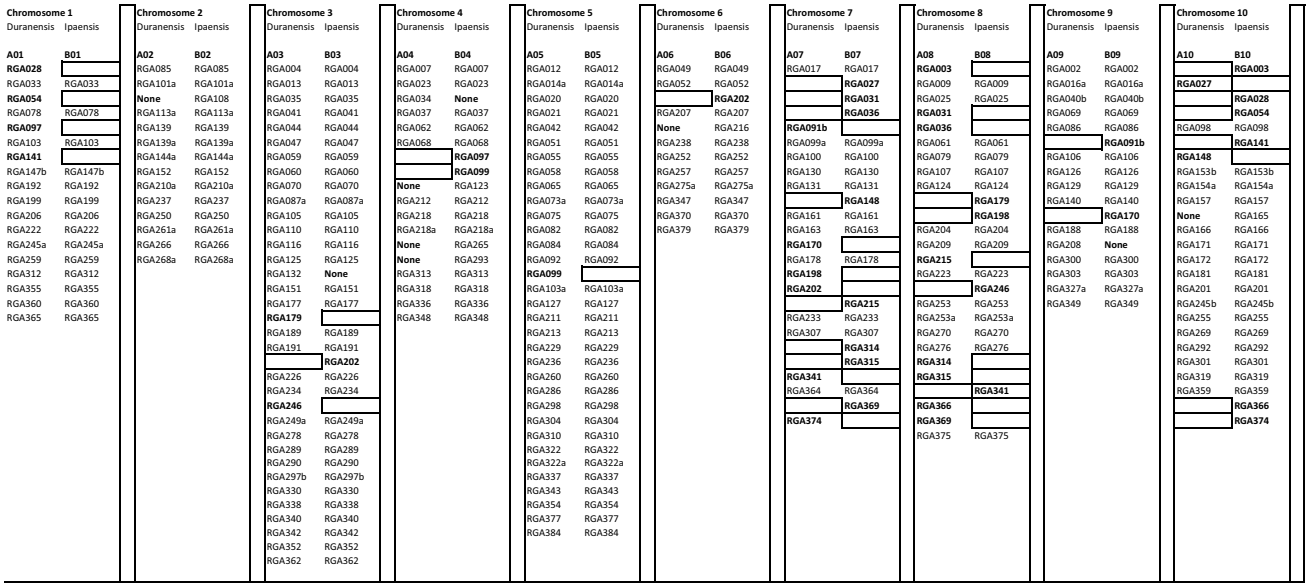
RGA ID	# SNPs	Size	# Var.	Diploids	RGA ID	# SNPs	Size	# Var.	Diploids
RGA002	0 SNPs	1653	3 Var.	I, D	RGA003	4 SNPs	712	Single	I, D
RGA013	0 SNPs	977	3 Var.	I, D	RGA004	6 SNPs	1736	Single	I, D
RGA016a	0 SNPs	975	3 Var.	I, D	RGA012	4 SNPs	810	Single	I, D
RGA020	0 SNPs	917	Single	I, D	RGA017	4 SNPs	597	Single	I, D
RGA023	0 SNPs	1334	Single	I, D	RGA021	6 SNPs	1551	Single	I, D
RGA026	0 SNPs	1551	Single	I, D	RGA025	3 SNPs	1191	Single	I, D
RGA027	0 SNPs	1630	2 Var.	I, D	RGA028	4 SNPs	677	Single	I, D
RGA031a	0 SNPs	1143	Single	I, D	RGA031	5 SNPs	876	Single	I, D
RGA040b	0 SNPs	1300	Single	I, D	RGA033	9 SNPs	1072	Single	I, D
RGA044	0 SNPs	714	Single	I, D	RGA035	7 SNPs	1000	2 Var.	I, D
RGA047	0 SNPs	1391	2 Var.	I, D	RGA037	5 SNPs	1363	Single	I, D
RGA051	0 SNPs	1242	2 Var.	I, D	RGA042	3 SNPs	983	Single	I, D
RGA052	0 SNPs	1711	3 Var.	I, D	RGA049	9 SNPs	745	Single	I, D
RGA055	0 SNPs	1122	Single	I, D	RGA054	10 SNPs	1602	3 Var.	I, D
RGA057	0 SNPs	919	Single	I, D	RGA062	6 SNPs	1677	Single	I, D
RGA058	0 SNPs	433	2 Var.	I, D	RGA065	2 SNPs	1084	Single	I, D
RGA059	0 SNPs	1059	Single	I, D	RGA069	4 SNPs	577	Single	I, D
RGA060	0 SNPs	569	Single	I, D	RGA073	11 SNPs	1204	2 Var.	I, D
RGA061	0 SNPs	964	3 Var.	I, D	RGA075	5 SNPs	1021	2 Var.	I, D
RGA084	0 SNPs	970	Single	I, D	RGA103	9 SNPs	1614	Single	I, D
RGA086	0 SNPs	453	3 Var.	I, D	RGA106	5 SNPs	1020	Single	I, D
RGA087a	0 SNPs	905	2 Var.	I, D	RGA107	8 SNPs	1386	Single	I, D
RGA091b	0 SNPs	851	Single	I, D	RGA110	11 SNPs	859	Single	I, D
RGA099	0 SNPs	1088	5 Var.	I, D	RGA123	16 SNPs	1302	Single	I
RGA100	0 SNPs	1131	2 Var.	I, D	RGA124	2 SNPs	923	Single	I, D
RGA101a	0 SNPs	1311	Single	I, D	RGA127	4 SNPs	415	Single	I, D
RGA102a	0 SNPs	1272	Single	I, D	RGA130	11 SNPs	1725	3 Var.	I, D
RGA108	0 SNPs	339	Single	I	RGA132	7 SNPs	598	Single	D
RGA113a	0 SNPs	885	Single	I, D	RGA139	2 SNPs	576	3 Var.	I, D
RGA116	0 SNPs	962	Single	I, D	RGA141	6 SNPs	1668	Single	I, D
RGA125	0 SNPs	1031	3 Var.	I, D	RGA146	5 SNPs	822	Single	I, D
RGA126	0 SNPs	1332	3 Var.	I, D	RGA151	3 SNPs	1143	Single	I, D
RGA129	0 SNPs	461	Single	I, D	RGA152	9 SNPs	1120	Single	I, D
RGA140	0 SNPs	964	2 Var.	I, D	RGA161	13 SNPs	1150	Single	I, D
RGA144a	0 SNPs	1653	Single	I, D	RGA162	3 SNPs	972	2 Var.	I, D
RGA157	0 SNPs	1624	2 Var.	I, D	RGA166	15 SNPs	1654	Single	I, D
RGA165a	0 SNPs	1189	Single	I, D	RGA177	16 SNPs	1658	4 Var.	I, D
RGA170	0 SNPs	1427	Single	I, D	RGA179	2 SNPs	1668	Single	I, D
RGA171	0 SNPs	1554	2 Var.	I, D	RGA189	6 SNPs	666	Single	I, D
RGA172	0 SNPs	1148	Single	I, D	RGA191	2 SNPs	1058	Single	I, D
RGA178	0 SNPs	1752	Single	I, D	RGA198	8 SNPs	641	Single	I, D
RGA197	0 SNPs	972	2 Var.	I, D	RGA202	6 SNPs	976	Single	I, D
RGA206	0 SNPs	1165	Single	I, D	RGA204	4 SNPs	1380	Single	I, D
RGA207	0 SNPs	1471	Single	I, D	RGA213	6 SNPs	816	2 Var.	I, D
RGA210a	0 SNPs	498	Single	I, D	RGA222	9 SNPs	940	4 Var.	I, D
RGA211	0 SNPs	1125	Single	I, D	RGA223	5 SNPs	1776	Single	I, D
RGA212	0 SNPs	1559	Single	I, D	RGA226	4 SNPs	793	2 Var.	I, D
RGA218	0 SNPs	615	2 Var.	I, D	RGA233	2 SNPs	1625	Single	I, D
RGA218a	0 SNPs	347	3 Var.	I, D	RGA234	11 SNPs	1747	Single	I, D
RGA224	0 SNPs	714	Single	I, D	RGA235	13 SNPs	614	3 Var.	I, D
RGA236	0 SNPs	735	Single	I, D	RGA246	2 SNPs	1701	Single	I, D

Table 2 (continued)

RGA ID	# SNPs	Size	# Var.	Diploids	RGA ID	# SNPs	Size	# Var.	Diploids
RGA237	0 SNPs	1712	3 Var.	I, D	RGA251	15 SNPs	1733	Single	I, D
RGA238	0 SNPs	1142	2 Var.	I, D	RGA260	7 SNPs	954	5 Var.	I, D
RGA240	0 SNPs	1153	3 Var.	I, D	RGA265	5 SNPs	736	Single	I, D
RGA252	0 SNPs	1742	Single	I, D	RGA286	8 SNPs	1349	Single	I, D
RGA253	0 SNPs	458	3 Var.	I, D	RGA312	12 SNPs	1702	Single	I, D
RGA253a	0 SNPs	683	2 Var.	I, D	RGA338	9 SNPs	1055	2 Var.	I, D
RGA266	0 SNPs	405	3 Var.	I, D	RGA345	11 SNPs	1652	3 Var.	I, D
RGA276	0 SNPs	810	3 Var.	I, D	RGA352	3 SNPs	843	Single	I, D
RGA278a	0 SNPs	1505	2 Var.	I, D	RGA355	5 SNPs	1337	2 Var.	I, D
RGA289	0 SNPs	1680	Single	I, D	RGA356	11 SNPs	1605	Single	I, D
RGA290	0 SNPs	737	3 Var.	I, D	RGA362	7 SNPs	919	Single	I, D
RGA292	0 SNPs	360	3 Var.	I, D	RGA364	6 SNPs	1458	4 Var.	I, D
RGA293	0 SNPs	232	Single	I	RGA366	11 SNPs	1441	2 Var.	I, D
RGA296	0 SNPs	353	Single	I, D	RGA375	2 SNPs	363	Single	I, D
RGA297b	0 SNPs	1199	2 Var.	I, D	RGA378	13 SNPs	1150	Single	I, D
RGA298	0 SNPs	1661	2 Var.	I, D	RGA379	9 SNPs	1550	Single	I, D
RGA300	0 SNPs	958	Single	I, D	RGA384	3 SNPs	1287	Single	I, D
RGA301a	0 SNPs	1422	Single	I, D	RGA073a	4 SNPs	1054	2 Var.	I, D
RGA319	0 SNPs	1685	2 Var.	I, D	RGA098	14 SNPs	1066	Single	I, D
RGA321a	0 SNPs	1573	Single	I, D	RGA121a	6 SNPs	1524	Single	I, D
RGA330	0 SNPs	1684	Single	I, D	RGA147b	4 SNPs	1095	Single	I, D
RGA337	0 SNPs	1521	Single	I, D	RGA153b	3 SNPs	602	4 Var.	I, D
RGA340	0 SNPs	758	2 Var.	I, D	RGA245b	10 SNPs	817	2 Var.	I, D
RGA341	0 SNPs	774	2 Var.	I, D	RGA007	1 SNP	1199	Single	I, D
RGA342	0 SNPs	872	Single	I, D	RGA144	1 SNP	1695	Single	I, D
RGA343	0 SNPs	560	Single	I, D	RGA147	4 SNPs	954	Single	I, D
RGA344	0 SNPs	1033	2 Var.	I, D	RGA148	1 SNP	1290	Single	I, D
RGA347	0 SNPs	1590	Single	I, D	RGA165	1 SNP	542	Single	I, D
RGA349	0 SNPs	1661	Single	I, D	RGA259	1 SNP	1619	2 Var.	I, D
RGA359	0 SNPs	1690	2 Var.	I, D	RGA268a	1 SNP	899	10 Var.	I, D
RGA360	0 SNPs	1410	Single	I, D	RGA270	1 SNP	1091	7 Var.	I, D
RGA363	0 SNPs	1355	Single	I, D	RGA336	1 SNP	1021	Single	I, D
RGA370	0 SNPs	1650	Single	I, D	RGA092a	4 SNPs	1164	Single	I, D
RGA374	0 SNPs	636	2 Var.	I, D	RGA099a	1 SNP	815	2 Var.	I, D
RGA377	0 SNPs	1524	3 Var.	I, D	RGA249b	3 SNPs	862	Single	I, D

peanuts may help to further ascertain gene-expression patterns that may better correlate genetic backgrounds as well incorporating environmental (biotic and abiotic) responses that will result in leaf spot resistance. Because of the conserved sequence domains, homologous sequence cloning as well as bioinformatics approach have identified a high number of potential peanut RGAs [21–23]. The resulting number of potential *R*-genes is a representative of the search databases and included 205,442 ESTs from *A. hypogaea* (allotetraploid) and other diploids such as 35,291 from *A. duranensis*, 32,787 from *A. ipaensis*, 6264 from *A. stenosperma*, 750 from *A. magna*, 400 from *A. appressipila*, and 280 from *A. Arabica*, 75 from *A. diogoi* (NCBI EST database, August, 2018). *R*-gene conversion is correlated with sequence identity, close physical proximity on the chromosome, gene orientation, and recombination rate [33]. From the point of view that cultivated peanut (*A. hypogaea*) came from two closest diploid progenitors (*A. duranensis* and *A. ipaensis*), the number of *R*-genes from cultivated peanuts may be similar to the diploids. Indeed from the peanut diploid sequencing projects, 345 and 397 *R*-gene candidates were identified in *A. duranensis* and *A. ipaensis* genotypes, respectively [18], which closely approximate the number of identified candidate *R*-genes (381) in cultivated peanuts across different taxon. Mapping *R*-genes from cultivated peanuts onto diploid genomes (A and B) revealed that chromosomes 1–6 and 9 have similar set of genes. Chromosomes 7, 8, and 10 showed significant

Table 3 Electronic mapping and placement of RGAs on *A. duranensis* and *A. ipaensis* chromosomes



Chromosome 1		Chromosome 2		Chromosome 3		Chromosome 4		Chromosome 5		Chromosome 6		Chromosome 7		Chromosome 8		Chromosome 9		Chromosome 10	
Duranensis	Ipaensis	Duranensis	Ipaensis	Duranensis	Ipaensis	Duranensis	Ipaensis	Duranensis	Ipaensis	Duranensis	Ipaensis	Duranensis	Ipaensis	Duranensis	Ipaensis	Duranensis	Ipaensis	Duranensis	Ipaensis
A01	**B01**	**A02**	**B02**	**A03**	**B03**	**A04**	**B04**	**A05**	**B05**	**A06**	**B06**	**A07**	**B07**	**A08**	**B08**	**A09**	**B09**	**A10**	**B10**
RGA028		RGA085	RGA085	RGA004	RGA004	RGA007	RGA007	RGA012	RGA012	RGA049	RGA049	RGA017	RGA017	**RGA003**		RGA002	RGA002		**RGA003**
RGA033	RGA033	RGA101a	RGA101a	RGA013	RGA013	RGA023	RGA023	RGA014a	RGA014a	RGA052	RGA052		**RGA027**	RGA009	RGA009	RGA016a	RGA016a	**RGA027**	
RGA054		**None**	RGA108	RGA035	RGA035	RGA034	**None**	RGA020	RGA020		**RGA202**		**RGA031**	RGA025	RGA025	RGA040b	RGA040b		**RGA028**
RGA078	RGA078	RGA113a	RGA113a	RGA041	RGA041	RGA037	RGA037	RGA021	RGA021	RGA207	RGA207		**RGA036**	**RGA031**		RGA069	RGA069		**RGA054**
RGA097		RGA139	RGA139	RGA044	RGA044	RGA062	RGA062	RGA042	RGA042	**None**	RGA216	**RGA091b**		**RGA036**		RGA086	RGA086	RGA098	RGA098
RGA103	RGA103	RGA139a	RGA139a	RGA047	RGA047	RGA068	RGA068	RGA051	RGA051	RGA238	RGA238	RGA099a	RGA099a	RGA061	RGA061		**RGA091b**		**RGA141**
RGA141		RGA144a	RGA144a	RGA059	RGA059		**RGA097**	RGA055	RGA055	RGA252	RGA252	RGA100	RGA100	RGA079	RGA079	RGA106	RGA106	**RGA148**	
RGA147b	RGA147b	RGA152	RGA152	RGA060	RGA060		**RGA099**	RGA058	RGA058	RGA257	RGA257	RGA130	RGA130	RGA107	RGA107	RGA126	RGA126	RGA153b	RGA153b
RGA192	RGA192	RGA210a	RGA210a	RGA070	RGA070	**None**	RGA123	RGA065	RGA065	RGA275a	RGA275a	RGA131	RGA131	RGA124	RGA124	RGA129	RGA129	RGA154a	RGA154a
RGA199	RGA199	RGA237	RGA237	RGA087a	RGA087a	RGA212	RGA212	RGA073a	RGA073a	RGA347	RGA347		**RGA148**		**RGA179**	RGA140	RGA140	RGA157	RGA157
RGA206	RGA206	RGA250	RGA250	RGA105	RGA105	RGA218	RGA218	RGA075	RGA075	RGA370	RGA370	RGA161	RGA161		**RGA198**		**RGA170**	**None**	RGA165
RGA222	RGA222	RGA261a	RGA261a	RGA110	RGA110	RGA218a	RGA218a	RGA082	RGA082	RGA379	RGA379	RGA163	RGA163	RGA204	RGA204	RGA188	RGA188	RGA166	RGA166
RGA245a	RGA245a	RGA266	RGA266	RGA116	RGA116	**None**	RGA265	RGA084	RGA084			**RGA170**		RGA209	RGA209	RGA208	**None**	RGA171	RGA171
RGA259	RGA259	RGA268a	RGA268a	RGA125	RGA125	**None**	RGA293	RGA092	RGA092			RGA178	RGA178	**RGA215**		RGA300	RGA300	RGA172	RGA172
RGA312	RGA312			RGA132	**None**	RGA313	RGA313	**RGA099**				**RGA198**		RGA223	RGA223	RGA303	RGA303	RGA181	RGA181
RGA355	RGA355			RGA151	RGA151	RGA318	RGA318	RGA103a	RGA103a			**RGA202**			**RGA246**	RGA327a	RGA327a	RGA201	RGA201
RGA360	RGA360			RGA177	RGA177	RGA336	RGA336	RGA127	RGA127				**RGA215**	RGA253	RGA253	RGA349	RGA349	RGA245b	RGA245b
RGA365	RGA365			**RGA179**		RGA348	RGA348	RGA211	RGA211			RGA233	RGA233	RGA253a	RGA253a			RGA255	RGA255
				RGA189	RGA189			RGA213	RGA213			RGA307	RGA307	RGA270	RGA270			RGA269	RGA269
				RGA191	RGA191			RGA229	RGA229				**RGA314**	RGA276	RGA276			RGA292	RGA292
					RGA202			RGA236	RGA236				**RGA315**	**RGA314**				RGA301	RGA301
				RGA226	RGA226			RGA260	RGA260			**RGA341**		**RGA315**				RGA319	RGA319
				RGA234	RGA234			RGA286	RGA286			RGA364	RGA364		**RGA341**			RGA359	RGA359
				RGA246				RGA298	RGA298				**RGA369**	**RGA366**					**RGA366**
				RGA249a	RGA249a			RGA304	RGA304			**RGA374**		**RGA369**					**RGA374**
				RGA278	RGA278			RGA310	RGA310					RGA375	RGA375				
				RGA289	RGA289			RGA322	RGA322										
				RGA290	RGA290			RGA322a	RGA322a										
				RGA297b	RGA297b			RGA337	RGA337										
				RGA330	RGA330			RGA343	RGA343										
				RGA338	RGA338			RGA354	RGA354										
				RGA340	RGA340			RGA377	RGA377										
				RGA342	RGA342			RGA384	RGA384										
				RGA352	RGA352														
				RGA362	RGA362														

Empty boxes represent a different chromosome location. Bold characters represent more than 1 chromosome locations

divergent between A and B genomes in this study, verifying what was observed by Bertioli et al. [18] that chromosomes 7 and 8 have undergone complex rearrangements in DNA segment exchange. The importance of identification of disease resistance gene through evaluation of *R*-genes will complement molecular mapping of different peanut genomes.

Out of the 381 potential *R*-gene candidates, 214 were identified and sequenced. From these, 72 (34%) were identified as RLKs and 25 (12%) as RLPs. RLKs and RLPs are PRRs that can interact in PAMP/MAMP to initiate signal transduction to elevate plant immunity response. The two molecules are structurally similar with a signal peptide at the N-terminus, extracellular domains to perceive the pathogen/microbial pattern as LRRs, and transmembrane to anchor RLK and RLP in the plasma membrane [2]. In contrast to RLKs, RLPs lack an intracellular kinase domain and do not independently transduce perceived signal downstream. Notable examples of are Flg22 and EFR, bacterial PAMPs for flagellin and elongation factor Tu (EF-Tu) sensing, that recruit FLS2 and BAK1 to activate kinase signaling cascade to initiate plant immune response [34, 35]. Receptor recognition of DNA, lipoproteins, peptidoglycans, and fungal chitin are also involved [36]. Plant resistance utilizing PRR activation has been thought to provide broad-spectrum resistance, but has not had much attention in breeding for disease resistance. In this study, 9 lectin-binding RLKs were identified (3 G-type and 6 L-type). In *Arabidopsis*, one of the largest class of RLKs are the L-type lectin receptor kinases (LecRKs) [37], and transgenic tobacco plants over-expressing *Arabidopsis* lectin receptor kinase gene (*LecRK-1.9* or *LecRK-1X.1*) show enhanced resistance to *Phytophthora* pathogens [38].

Another class designated as "other" variations included 85 (40%) with STK domain. These included RGAs 016a and 222 that code for a *pto*-interacting protein 1-like; and RGAs 091b, 140, 327a which code for the corresponding *pti1*-like tyrosine-protein kinase important in disease resistance signaling mechanism in tomato [39]. In the mitogen-activated protein kinase (MAPK) signal transduction cascade, RGAs 014a, 092, 147b, 172, 237, and 377 code for mitogen-activated protein kinase kinase kinase; and RGA078 codes for a mitogen-activated protein kinase kinase. Plant MAPK cascades regulate a wide range of responses including stress, hormone regulation, innate immunity, and development [40]. RGA105 and 116 code for polygalacturonase-inhibiting protein gene 2. Expression of a polygalacturonase-inhibiting protein gene 2 (*MsPGIP2*) in alfalfa confer resistance to common leaf spot [41]. However in this study, RGA 116 was observed to be down-regulated compared to susceptible control, FR458. This may be a target for overexpression in plants to confer disease resistance.

Only 30 (14%) are represented by 1 CNL and 29 TNL combined. A large number (22 RGAs) codes for TMV resistance protein N-like and gene-expression may confer virus resistance [42]. Sequence variations for N-like proteins in peanut may recognize common Avr proteins in different viruses [43]. CNL and TNL are receptors that are regulated in ETI response, which can be stronger and longer than PTI response. In terms of *R*-gene evolution and transmission, it is theorized that *R*-genes are duplicated, rearranged, and/or mutated with synonomous

Fig. 2 **a, b** Relative gene-expression levels of RGAs. All samples were first normalized to *Actin* (EZ723877) as an internal control then transformed data were normalized with FR458 and compared to the other peanut genotypes to obtain relative gene-expression levels

or non-synonomous insertions/deletions (indels) or single nucleotide variations (SNPs) [44]. In this study, the majority of identified *R*-genes were observed to have low DNA polymorphism. Out of the 172 RGAs cloned and sequenced, 86 (50%) showed no SNPs and another 86 (50%) showed fewer than 16 SNPs in expressed transcripts

ranging from 232 to 1776 bp. Only four RGAs with different size indels (3–9 bp), representing synonomous and inframe indels, were discovered. In peanuts, expressed genes with indels can be associated with disease resistance [12] and peanut agronomic traits [45]. In rice, a major rice blast resistance gene *Pi54* (Pikh), is associated with an NBS–LRR containing protein with a 144-bp insertion/deletion (indel) [46]. The rest the *R*-genes showed high SNP polymorphism which may represent more than one allele for each transcript. Sequences with multiple variants, such as SNPs or indels, can code for different proteins and perhaps add new complementary function. For cultivated peanuts, these 214 RGAs represent transcript expression levels sufficient to be observed on agarose gel-electrophoresis analysis. Differences in the levels of gene-expression of RGAs can be associated to different levels of disease resistance, identifying potential disease resistance genes. Since the majority of the *R*-genes in study belong to PRRs and others with STK domains (~ 80%), it is difficult to draw a significant conclusion about all *R*-gene evolution in peanuts. In a study analyzing molecular phylogeny and evolution in legumes, *R*-genes were observed to undergo purifying selection instead of positive selection [47]. Initial low-frequency of genes introduced by random recombination may be lost (perhaps due lack of disease pressures, artificial selection by domestication, or fitness costs). The introgression wild peanut species may provide a novel source of disease resistance genes since some diploid peanuts have been observed to be more disease resistant than cultivated peanuts [48].

Disease resistance in plants is complex and involves a balance between disease responses and plant productivity. Comparison of disease susceptible versus more tolerant genotypes identified a group of up-regulated and down-regulated *R*-genes that are potential targets for molecular breeding or applications in biotechnology. Research provides valuable information to understand disease resistance mechanism(s) relating to the gene-expression of *R*-genes in cultivated peanut and provides gene targets to develop disease resistant peanut varieties.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

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