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Plant Resistance to TSWV and Seed Accumulation of Resveratrol within Peanut Germplasm and its Wild Relatives in the US Collection

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Abstract: Biotic and abiotic stress may induce peanut plants to produce a high amount of resveratrol. The relationship between an individual plant's response to biotic stress caused by *Tomato spotted wilt virus* (TSWV) and the accumulation of resveratrol in the seed was investigated. Twenty peanut accessions and six wild relatives were selected from the US peanut germplasm collection and planted with two replicates. Individual plant response to natural-TSWV infection was observed and recorded in the field. Leaf tissues from each individual plant were collected and tested by an Enzyme-Linked Immunosorbent Assay (ELISA) using specific antiserum for TSWV. Seeds harvested from individual plants were used for quantification of resveratrol by High Performance Liquid Chromatography (HPLC) analysis. Extensive resveratrol variation in the seeds was detected among TSWV negative and positive plants. Among the accessions evaluated in this study, the specific genotype of each individual definitely played a major role on the capability for synthesis and accumulation of resveratrol. However, the synthesis and accumulation of resveratrol within an accession may not only be affected by a plant's response to TSWV, but also by other biotic and abotic stress that an individual plant encounters in its environment.

Key words: *A. hypogaea* L., *Tomato spotted wilt virus*, ELISA, resveratrol content, HPLC

INTRODUCTION

Peanut, *Arachis hypogaea* L. (2n = 4x = 40) is one of the five most important oilseed crops cultivated worldwide. Peanut seeds are nutritious containing about 44-56% oil and 22-30% protein (Pancholy *et al*., 1978). Due to their high oil and protein content, peanuts are mainly used for edible oil production and high-protein food consumption, especially in developing countries where there is limited access to protein sources (Singh and Singh, 1991). Additionally, peanut seeds also contain useful phytochemicals such as flavonoids, folic acids (also known as vitamin B_9), tocopherols (vitamin E) and *trans*-Resveratrol (*trans*-3,4,5′-trihydroxystilbene). Consumption of foods containing these phytochemicals is believed to be beneficial to human health (Jang *et al*., 1997; Ross and Kasum, 2002; Alper and Mattes, 2003; Jonnala *et al*., 2006). The *trans*-Resveratrol can greatly contribute to human health due to its antioxidant, anti-inflammatory, anticancer activities, as well as chemopreventive, cardioprotective and estrogenic effects (Baur and Sinclair, 2006).

The *trans*-Resveratrol in peanut cultivars and products has been previously quantified by HPLC (Sobolev and Cole, 1999; Sanders *et al*., 2000). A recent study (Sobolev *et al*., 2007) demonstrated that seeds in the pods damaged by lesser corn borer from some genotypes contain a higher amount of phytoalexins (including *trans*-Resveratrol and other resveratrol derivatives) than other genotypes. *Tomato spotted wilt virus* (genus *Tospovirus*, family Bunyaviride, TSWV) is a serious threat to peanut production and causes a significant yield loss worldwide. Annual peanut yield losses due to TSWV were estimated to be about $40-100 million in Georgia alone (Jain *et al*., 1998; Culbreath *et al*., 2003). However, there was no evidence found that stilbene phytoalexins are directly involved in peanut resistance to TSWV. So far, there are not any effective molecular tools (DNA markers and/or biochemical markers) available for rapidly selecting resistance to TSWV in peanut breeding programs. Investigating the relationship between plant response to TSWV and resveratrol synthesis and accumulation may provide information on developing biochemical markers for the selection of resistance to TSWV.

Peanut germplasm accessions have been evaluated for resistance to TSWV in diploid and tetraploid species (Lyerly *et al*., 2002; Wang *et al*., 2007) and some resistant

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accessions have been identified. Breeding lines highly resistant to TSWV have also been developed by crossing and selecting interspecific progenies (Holbrook *et al*., 2003). Peanut germplasm accessions have also been screened by HPLC for resveratrol content in seeds and at least a ten-fold difference (ranging from 0.125 to 1.626 μg g^{-1}) has been identified among accessions (Wang *et al*., 2008). Evaluating the plant response to TSWV by field observation, confirming presence or absence of infection by ELISA and quantifying the resveratrol content in seeds by HPLC using the same set of peanut plants may help to reveal some clue about the relationship between plant response to TSWV and seed accumulation of resveratrol in peanut. Therefore, the objectives of this study were to (1) evaluate the response of peanut accessions and their wild relatives to TSWV, (2) determine the resveratrol content in peanut seeds from TSWV-infected and -uninfected plants, (3) examine the variation in genetic potential of accessions to produce resveratrol and (4) investigate whether there is an association between plant response to TSWV and seed accumulation of resveratrol in peanut.

MATERIALS AND METHODS

Twenty peanut accessions within *A. hypogaea* L. (2n = 4x = 40) and six diploid wild relatives (2n = 2x = 20) were selected from the US germplasm collection. Two subspecies (*fastigiata* and *hypogaea*) and three botanic varieties (var. *fastigiata*, var. *hypogaea* and var. *vulgaris*) were included in the 20 accessions. The six wild relatives included in this study consisted of one accession from each of the four species (*A. cardenassi* Krapov and W. C. Greg, *A. diogoi* Hoehne, *A. kempff-mercadoi* Krapov and *A. magna* Krapov) and two accessions from *A. stenosperma* Krapov and W. C. Greg. The accession number, species name, ploidy level, accession identifier and collection site of experimental materials used are listed in Table 1. Sixty peanut seeds from each accession were planted in a ten-foot plot with two replicate plots in 2008 at Byron, Georgia. The selected accessions were exposed to natural TSWV infection. When the TSWV symptoms were fully developed four months after planting, the whole plot was scored for plant response to TSWV from resistant to susceptible (ranked from 0 to 5) according to a similar scale for scoring leaf spot (Chiteka *et al*., 1988) by three evaluators. Then, two to four individual plants within peanut accessions were randomly labeled for leaf tissue and seed collection.

Fresh leaf tissues from each labeled plant were collected at two different times (three weeks apart) and used for TSWV detection. The detection by an alkaline phosphatase-based Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) was conducted following the method (Pinnow *et al*., 1990) in 2008 in Griffin, Georgia. Finished plates were read at 405 nm on a Molecular Devices E-max plate reader.

Seeds were harvested from each ELISA-assayed individual plant. *Tomato spotted wilt virus* (TSWV) may affect seed-coat color or seed weight. Therefore, seed-coat colors and seed weight (g/100 seeds) were recorded. In order to evaluate the effect of TSWV

Table 1: Selected peanut accessions and its wild relatives

PI No.	Species	Ploidy level	Identifier	Collection site
PI 338280	*A. stenosperma* Krapov and W.C. Greg	2n = 2x = 20	410	Brazil
PI 497578	*A.* stenosperma Krapov and W.C. Greg	2n = 2x = 20	VMoGeSv 7377	Brazil
PI 468331	*A. kempff-mercadoi* Krapov	2n = 2x = 20	GK SPScZ 30085	Bolivia
PI 468337	*A. magna* Krapov	2n = 2x = 20	GK SSC 30092	Bolivia
PI 476012	*A. cardenassi* Krapov and W.C. Greg	2n = 2x = 20	KSSc 36033	Bolivia
PI 468354	*A. diogoi* Hoehne	2n = 2x = 20	GK PSc 30106	Paraguay
Geogria G	*A. hypogaea* L.	2n = 4x = 40	Georgia Green	US
PI 159786	*A. hypogaea* L.	2n = 4x = 40	n/a	Senegal
PI 247372	*A. hypogaea* L.	2n = 4x = 40	Philippine white	Gambia
PI 296550	*A. hypogaea* L.	2n = 4x = 40	178	Israel
PI 313129	*A. hypogaea* L.	2n = 4x = 40	101	China
PI 319768	*A. hypogaea* L.	2n = 4x = 40	1066-20	Israel
PI 331297	*A. hypogaea* L.	2n = 4x = 40	132	Argentina
PI 331314	*A. hypogaea* L.	2n = 4x = 40	149	Argentina
PI 337373	*A. hypogaea* L.	2n = 4x = 40	Negro Grande	Paraguay
PI 339960	*A. hypogaea* L.	2n = 4x = 40	n/a	Argentina
PI 442768	*A. hypogaea* L.	2n = 4x = 40	P34/6/2	Zimbabwe
PI 468261	*A. hypogaea* L. var. *hypogaea*	2n = 4x = 40	US 66	Bolivia
PI 471952	*A. hypogaea* L.	2n = 4x = 40	31/7/10	Zimbabwe
PI 493582	*A. hypogaea* L. var. *fastigiata*	2n = 4x = 40	RCM 274	Argentina
PI 493965	*A. hypogaea* L. var. *fastigiata*	2n = 4x = 40	RCM 657	Argentina
PI 494054	*A. hypogaea* L. var. *vulgaris* Harz	2n = 4x = 40	RCM 746	Argentina
PI 497255	*A. hypogaea* L. var. *hypogaea*	2n = 4x = 40	803-1	Argentina
PI 497422	*A. hypogaea* L. var. *hypogaea*	2n = 4x = 40	US 830-2	Bolivia
PI 602067	*A. hypogaea* L.	2n = 4x = 40	K-493	China
PI 628560	*A. hypogaea* subsp. *fastigiata* Waldron	2n = 4x = 40	WTS 38	Ecuador

infection on seed accumulation of resveratrol, the collected seeds within an accession were chosen in pairs from positive and negative plants which had been confirmed by ELISA. Seeds from the paired plants (positive- and negative-ELISA results within an accession) were used for quantification of resveratrol by High Performance Liquid Chromatography (HPLC) analysis.

High performance liquid chromatography analysis for resveratrol was conducted in 2008 in Griffin, Georgia and followed the method described by Wang and Pittman (2008). Approximately eight grams of air-dried seeds were ground into a fine powder in a coffee blender. Ground seed tissue (3 g) was transferred into 50 mL Falcon tubes and homogenized with 9 mL of 80% ethanol using a Power Gen 125 homogenizer (Fisher Scientific, Waltham, MA). The homogenized samples were centrifuged at 12,000 rpm for 3 min. Two milliliter of supernatant were taken and cleaned by solid-phase extraction using Poly-Prep chromatography column [0.8×4 cm, (Bio-Rad, Hercules, CA)] packed with ~ 1 mL mixture (1:1 w/w) of Al_2O_3 (EM Industries Inc., Hawthorne, NY) and silica gel 60 RP-18 (EMD Chemicals Inc., Gibbstown, NJ). The packed column was conditioned with 80% ethanol. The supernatant was applied to the equilibrated column and the effluent was collected into a 4 mL vial. The column was washed with an additional 2 mL of 80% ethanol and the effluent was collected into the same vial. The collected solvent was evaporated at 50ºC to dryness with a nitrogen gas stream. The extracted compounds were dissolved in 1 mL of 20% acetonitrile and filtered (at 0.45 µm filter) prior to injection for HPLC analysis. Separation of metabolites was performed on RP-HPLC system (Agilent 1100 series) using a C_{18} column [4.6 mm×150 mm, 5 µm, (Agilent Technologies, Santa Clara, CA)] at 40ºC with a binary pump and autosampler. The mobile phase consisted of A: filtered sterile water containing 0.1% formic acid at pH 2.5 and B: HPLC-grade acetonitrile. The flow rate was 1.5 mL min^{-1} with the following gradient: 10% B for 2 min, 10-30% B for 8 min, 30% B for 1 min, followed by a column wash at 95% B for 6 min and 10% B for 9 min before next injection. The volume for sample injection was 30 µL and the analytes were monitored with a Diode-Array Detector (DAD) at 310 nm absorbance. The *trans*-Resveratrol in the extract of each accession was quantified at 310 nm by reference to the peak area of an external authentic standard of resveratrol. Two replicated samples were tested for determination of *trans*-Resveratrol concentration. Two sample extractions were conducted for each replicate after the grinding step. The average of the two extractions per sample was used for data analysis. Regression analysis was conducted using statistical analysis system.

RESULTS

Field evaluation using visual examination for plant response to TSWV: The plot was scored from 0 to 5 representing a range of resistant to susceptible plants, respectively according to the similar scale for scoring leaf spot (Chiteka *et al*., 1988). As an example, symptoms caused by TSWV infection are shown in Fig. 1. Peanut plant number 67 (scored as 0) was from a resistant plot (PI 412768). There were no visible TSWV symptoms observed. Peanut plant number 59 and 6 (both were scored as 4) were from susceptible plots (PI 331297 and PI 494054). Clear symptoms (characteristic ring-spots in the leaves, yellowing, stunting and necrosis) were observed on both plants. The plot scores from two replicates are summarized in Table 2. All six peanut wild relatives showed high resistance to TSWV and the results from two replicates were consistent (Table 2, Fig. 1). Among twenty peanut accessions, only two accessions (PI 319768 and PI 442768) showed high resistance to TSWV while seven accessions (PI 331297, PI 331314, PI 471952, PI 494054, PI 602067 and PI 628560) showed high susceptibility to TSWV. The results for these nine accessions were consistent across two replicates. However, the response to TSWV from the other eleven peanut accessions was inconsistent between the two replicates (Table 2). To further confirm the field plot score, 125 individual plants were selected from these twenty peanut accessions. The leaf tissues from these individual plants were collected for testing by ELISA.

Evaluation using ELISA for plant response to TSWV infection: Enzyme-linked immunosorbent assay testing for TSWV was conducted twice (ELISA 1 and ELISA 2) from the leaf tissues collected at two different developmental stages and the test results are summarized in Table 3. An ELISA value >0.1 was classified as positive (P) for TSWV; whereas, an ELISA value <0.1 was classified as negative (N). Among 125 plants, 114 plants (91.2%) showed consistent results (either negative or positive) from two separate ELISA tests. However, 11 plants (8.8%) showed inconsistent results from the two ELISA values (i.e., positive to negative or negative to positive between the replicates). For example, four plants from accession PI 296650 were evaluated by ELISA. All of them were negative and ELISA values were very low (all of them were <0.03, Table 3). Therefore, this peanut accession can be confidently classified as uninfected by TSWV based on the ELISA value. Eight plants from the accession PI 247372 were evaluated by ELISA. Four of the plants showed positive values (with some values >2.0, much higher than 0.1) and four of them showed negative values. This accession can be confidently

Fig. 1: Plant response from different species to TSWV infection. The pictures were taken for four-month old plants. Plant number 67, 59 and 6 are classified as *A. hypogaea* L. (a) The accession PI 442768 (plant number 67 without any symptoms) was resistant to TSWV infection, (b) The accession PI 331297 (plant number 59) was highly susceptible to TSWV infection, © The accession PI 494054 (plant number 6) was highly susceptible to TSWV, (d) The accessions of PI 497578 (*A. stenosperma*), (e) PI 463854 (*A. diogoi*), (f) PI 476012 (*A. cardenassi*), (g) PI 468331 (*A. kempff-mercadoi*) and (h) PI 468337 (*A. magna*) were resistant to TSWV infection. R: Resistant, S: Susceptible

Table 2: Field evaluation using visual examination to score for TSWV infection at replicate I and II

PI No.	Species	I-1	I-2	I-3	I-4	PS I	II-1	II-2	II-3	II-4	PS II	PS I + II
PI 338280	*A. stenosperma* Krapov and W.C. Greg		0					0	0			
PI 497578	*A. stenosperma* Krapov and W.C. Greg		0					0	0			
PI 468331	*A. kempff-mercadoi* Krapov			0					0	0		
PI 468337	*A. magna* Krapov				0					0	0	
PI 476012	*A. cardenassi* Krapov and W.C. Greg		0					0	0			
PI 468354	*A. diogoir* Hoehne				0					0	0	
Georgia G	*A. hypogaea* L.	262	158			0	18	216			0.5	0-0.5
PI 159786	*A. hypogaea* L.	260	47	5	256	1.5	176	104			0	0-1.5
PI 247372	*A. hypogaea* L.	251	253	93	227	0	25	39	113	124	1	0-1.0
PI 296550	*A. hypogaea* L.	139	106			0	31	50			1	0-1.0
PI 313129	*A. hypogaea* L.	33	82	119	121	2	110				0	0-2.0
PI 319768	*A. hypogaea* L.	246	268			0	211	146			0	0
PI 331297	*A. hypogaea* L.	59	61	15	56	4	276	219	112	169	0.5	0.5-4.0
PI 331314	*A. hypogaea* L.	4	269	244	245	0.5	19	17	279	150	1	0.5-1.0
PI 337373	*A. hypogaea* L.	20	230			1	122	131			0	0-1.0
PI 339960	*A. hypogaea* L.	35	46	36	4	2	225	147			0	0-2.0
PI 442768	*A. hypogaea* L.	67	68			0	218	116			0	0
PI 468261	*A. hypogaea* L. var. *hypogaea*	118	111			0	14	299	259	24	3	0-3.0
PI 471952	*A. hypogaea* L.	265	264	65	263	3	247	289	75	149	0.5	0.5-3.0
PI 493582	*A. hypogaea* L. var. *fastigiata*	272	273	21	3	1.5	249	105	94	297	2.5	1.5-2.5
PI 493965	*A. hypogaea* L. var. *fastigiata*	54	57	64	45	2	115	144			0	0-2.0
PI 494054	*A. hypogaea* L. var. *vulgaris* Harz	6	32	289	243	4	16	22	86	163	1.5	1.5-4.0
PI 497255	*A. hypogaea* L. var. *hypogaea*	55	66	60	275	2	224	153			0	0-2.0
PI 497422	*A. hypogaea* L. var. *hypogaea*	12	26	240	242	1	132	126			0	0-1.0
PI 602067	*A. hypogaea* L.	271	62	77	127	4	221	114	249	142	2	2.0-4.0
PI 628560	*A. hypogaea* subsp. *fastigiata* Waldron	254	29	108	123	1	13	29	220	156	1	1

For each accession, if symptoms were observed, four plants would be labeled (I- or II- 1, 2, 3, and 4) as series number. Plot Score (PS) was scaled from 0 to 5 (from resistant to susceptible)

classified as moderately susceptible to TSWV based on the ELISA values. Among twenty peanut accessions assayed by ELISA, only two accessions (PI 296550 and PI 319768) were identified as highly resistant to TSWV for

Table 3: Lab. evaluation using ELISA results for the scored individual plants from two replicates

PI No.	Plant No. (I)	ELISA 1	ELISA 2	P/N	Plant No. (II)	ELISA 1	ELISA 2	P/N
Georgia G	262	0.006 N	0.002 N	N	18	3.447 P	2.553 P	P
	158	0.026 N	0.009 N	N	216	0.006 N	2.108 P	P/N
PI 159786	260	1.811 P	0.383 P	P	176	0.012 N	0.019 N	N
	47	0.006 N	0.008 N	N	104	0.005 N	0.009 N	N
	5	2.329 P	0.211 P	P	X			
	256	0.000 N	0.012 N	N	X			
PI 247372	251	3.428 P	2.060 P	P	25	3.443 P	2.425 P	P
	253	3.346 P	1.852 P	P	39	0.348 P	1.294 P	P
	93	0.007 N	0.008 N	N	113	0.012 N	0.009 N	N
	227	0.056 N	0.007 N	N	124	0.009 N	0.007 N	N
PI 296550	139	0.009 N	0.025 N	N	31	0.007 N	0.007 N	N
	106	0.005 N	0.013 N	N	50	0.016 N	0.010 N	N
PI 313129	33	0.005 N	0.009 N	N	110	0.007 N	0.007 N	N
	82	0.007 N	0.011 N	N	X			
	119	0.530 P	2.170 P	P	X			
	121	2.411 P	0.345 P	P	X			
PI 319768	246	0.002 N	0.007 N	N	211	0.005 N	0.003 N	N
	268	0.001 N	0.010 N	N	146	0.003 N	0.002 N	N
PI 331297	59	0.219 P	0.259 P	P	276	3.562 P	2.680 P	P
	61	0.006 N	0.007 N	N	219	0.036 N	0.002 N	N
	15	3.411 P	1.647 P	P	112	0.008 N	0.010 N	N
	56	0.011 N	0.009 N	N	169	0.017 N	0.003 N	N
PI 331314	4	1.075 P	0.113 P	P	19	0.000 N	0.003 N	N
	269	0.794 P	0.794 P	P	17	0.975 P	0.294 P	P
	244	0.009 N	0.154 P	P/N	279	1.009 P	0.502 P	P
	245	0.009 N	0.001 N	N	150	0.006 N	0.005 N	N
PI 337373	20	3.345 P	0.005 N	P/N	122	0.002 N	0.005 N	N
	230	0.041 N	0.005 N	N	131	0.010 N	0.011 N	N
PI 339960	35	0.017 N	0.014 N	N	225	0.046 N	0.003 N	N
	46	1.264 P	0.262 P	P	147	0.005 N	0.007 N	N
	36	0.005 N	0.011 N	N	X			
	41	1.036 P	x	P	X			
PI 442768	68	0.012 N	0.013 N	N	218	0.667 P	0.269 P	P
	67	0.008 N	0.009 N	N	116	0.016 N	0.008 N	N
PI 468261	118	0.007 N	0.009 N	N	14	0.711 P	0.333 P	P
	111	0.008 N	0.012 N	N	299	0.004 N	0.016 N	N
	X				259	0.007 N	0.008 N	N
	X				24	3.312 P	1.768 P	P
PI 471952	265	1.954 P	1.302 P	P	247	0.006 N	0.010 N	N
	264	0.006 N	0.339 P	P/N	289	3.275 P	1.738 P	P
	65	0.008 N	0.164 P	P/N	75	0.659 P	0.129 P	P
	263	0.004 N	0.004 N	N	149	0.004 N	0.004 N	N
PI 493582	272	2.786 P	0.008 N	P/N	294	0.008 N	0.022 N	N
	273	0.014 N	0.011 N	N	105	0.674 P	0.459 P	P
	21	2.343 P	0.595 P	P	94	0.437 P	0.372 P	P
	3	0.016 N	0.004 N	N	297	0.010 N	0.009 N	N
PI 493965	54	0.367 P	0.172 P	P	115	0.019 N	0.009 N	N
	64	0.937 P	0.115 P	P	144	0.007 N	0.005 N	N
	57	0.020 N	1.519 P	P/N	X			
	45	0.002 N	0.011 N	N	X			
PI 494054	6	0.201 P	0.150 P	P	16	0.023 N	0.005 N	N
	243	0.006 N	0.000 N	N	22	3.505 P	3.198 P	P
	289	0.257 P	3.177 P	P	86	3.359 P	1.329 P	P
	32	0.013 N	0.007 N	N	163	0.020 N	0.006 N	N
PI 497255	55	0.149 P	0.010 N	P/N	224	0.058 N	0.009 N	N
	66	0.188 P	0.096 N	P/N	153	0.013 N	0.006 N	N
	60	0.013 N	0.011 N	N	X			
	275	0.019 N	0.031 N	N	X			
PI 497422	12	3.373 P	1.412 P	P	132	0.018 N	0.011 N	N
	26	2.569 P	0.003 N	P/N	126	0.008 N	0.008 N	N
	240	0.000 N	0.005 N	N	X			
	242	0.007 N	0.000 N	N	X			
PI 602067	271	3.420 P	0.693 P	P	221	0.581 P	0.120 P	P
	62	0.010 N	0.019 N	N	114	0.019 N	0.010 N	N
	77	0.948 P	0.223 P	P	249	3.413 P	1.568 P	P
	127	0.013 N	0.006 N	N	142	0.003 N	2.713 P	P/N
PI 628560	254	3.440 P	1.804 P	P	13	0.026 N	0.007 N	N
	29	3.009 P	1.109 P	P	29	3.265 P	0.801 P	P
	108	0.004 N	0.009 N	N	220	0.033 N	0.007 N	N
	123	0.010 N	0.008 N	N	156	0.004 N	0.023 N	N

ELISA value >0.1 is considered as positive (P) and <0.1 as negative (N). I and II were replicates. x indicates that no leaf tissue was available for collection

all plants in two replications. The other eighteen accessions were identified as more or less susceptible to TSWV. Four plants from Georgia Green (a major peanut cultivar grown in the Southeast United States) were tested by ELISA and one of them was identified as positive (Table 3). Due to co-evolution of plants and viruses, the high disease pressure may result in losses of resistance to TSWV in Georgia Green.

Comparison of ELISA results with field observations: The peanut accessions response to TSWV infection may be better evaluated by a combination of results from ELISA and field observations. Based on the results determined by ELISA (negative or positive), 95 and 90% of the field observation was confirmed by ELISA assay for the replicate I and II, respectively. For example, the accession of PI 319768 was scored as highly resistant to TSWV (0) from two replicates. Four plants from this accession were evaluated by ELISA and all the plants were classified as negative. The results from both ELISA and field observations (Table 4) confirm that PI 319768 is highly resistant to TSWV. However, the accession PI 442768 was scored as highly resistant to TSWV (0) from two replicates in the field observation; whereas, one plant was detected as positive (susceptible to TSWV) by ELISA among four plants from this accession. In the early stages of viral infection, the symptoms may not be easily identified by field observation, but the virus titer may be high enough to be detected by ELISA. In combination of ELISA and field observation, the accession PI 442768 can only be classified as moderately resistant to TSWV. There was a slight discrepancy between the results of the two replicate field observations for accession PI 296550. PI 296550 was scored as highly resistant to TSWV (0) in replicate I; whereas, it was scored as resistant to TSWV (1) in replicate II. However, from four plants assayed by ELISA, all plants were classified as negative (highly resistant to TSWV). There are two possible reasons to explain the difference: one is the field observation for this accession is not accurate and another is not enough plants were assayed by ELISA. To classify this accession correctly, more plants need to be observed in the field and assayed by ELISA. Overall, ELISA results confirm the field observation for most of the accessions investigated. The developmental stages of the plants could affect the results from both field observation and ELISA.

Response to TSWV and amount of resveratrol accumulation: After TSWV infection, the seed production from some infected plants was severely reduced (data not shown). Even though all labeled plants were dug from the soil for seed collection, there were not enough seeds collected for resveratrol measurement for some accessions. These accessions were highly susceptible to TSWV. In total, there were fifteen pairs of plants (negative paired with positive) which produced enough seeds for resveratrol measurement. The resveratrol results from HPLC with ELISA data are listed in Table 5. Among thirty plants, plants No. 25 and 127 accumulated extremely high amounts of resveratrol (13.735 and 11.318 μg g^{-1}), at least twenty-times higher than their paired counterpart (0.546 μg g^{-1} for plant No. 113 and 0.141 μg g^{-1} for plant No. 77). Intriguingly, plant No. 25 was identified as positive for TSWV; whereas, plant No. 127 was identified as negative by ELISA (Table 5). The increase in resveratrol accumulation cannot be explained only by TSWV infection.

Table 4: ELISA results and field obser√ation for confirmation

PI No.	ELISA (I)	PS (I)	Confirmation	ELISA (II)	PS (II)	Confirmation
Georgia G	0P/4N	0	√	3P/1N	0.5	√
PI 159786	4P/4N	1.5	√	0P/4N	0	√
PI 247372	4P/4N	0	×	4P/4N	1	√
PI 296550	0P/4N	0	√	0P/4N	1	×
PI 313129	4P/4N	2	√	0P/2N	0	√
PI 319768	0P/4N	0	√	0P/4N	0	√
PI 331297	4P/4N	4	√	2P/6N	0.5	√
PI 331314	5P/3N	0.5	√	4P/4N	1	√
PI 337373	1P/3N	1	√	0P/4N	0	√
PI 339960	3P/4N	2	√	0P/4N	0	√
PI 442768	0P/4N	0	√	2P/2N	0	×
PI 468261	0P/4N	0	√	4P/4N	3	√
PI 471952	4P/4N	3	√	4P/4N	0.5	√
PI 493582	3P/5N	1.5	√	4P/4N	2.5	√
PI 493965	5P/3N	2	√	0P/4N	0	√
PI 494054	4P/4N	4	√	4P/4N	1.5	√
PI 497255	2P/6N	2	√	0P/4N	0	√
PI 497422	3P/5N	1	√	0P/4N	0	√
PI 602067	4P/4N	4	√	5P/3N	2	√
PI 628560	4P/4N	1	√	2P/6N	1	√
Percentage			95			90

√: The results from ELISA and plot score (PS) confirmed each other. ×: The results from ELISA and PS did not confirm each other. PS (I) and PS (II) were from replicate I and II, respectively

Fig. 2: Plants with stress and TSWV infection. The pictures were taken for four-month old plants. (a) The accession PI 442768 (plant number 67 without any symptoms) was resistant to TSWV infection, (b) The accession PI 331297 (plant number 59) was highly susceptible to TSWV infection, © The accession PI 494054 (plant number 6) was highly susceptible to TSWV, (d) The accessions of PI 497578 (*A. stenosperma*), (e) PI 463854 (*A. diogoi*), (f) PI 476012 (*A. cardenassi*), (g) PI 468331 (*A. kempff-mercadoi*) and (h) PI 468337 (*A. magna*) were resistant to TSWV infection. Resveratrol content in seeds from the plant harvested is given underneath its picture. R: Resistant, S: Susceptible

Table 5: Comparison between ELISA data and resveratrol accumulation in seeds from single plants in pairs

PI No.	Plant No.	Seed weight	ELISA 1	ELISA 2	Average	Resveratrol 1	Resveratrol 2	Average
PI 247372	93	46.30	0.007 N	0.008 N	0.0075	0.608	0.554	0.5810
	251	50.20	3.428 P	2.060 P	2.7440	1.944	1.838	1.8910
	113	41.90	0.012 N	0.009 N	0.0105	0.549	0.543	0.5460
	25	35.85	3.443 P	2.425 P	2.9340	13.146	14.324	13.7350
PI 331314	244	36.90	0.009 N	0.154 P	0.0815	0.32	0.312	0.3160
	4	36.10	1.075 P	0.113 P	0.5943	0.122	0.107	0.1140
PI 313129	82	62.40	0.007 N	0.011 N	0.0090	0.108	0.098	0.1030
	119	50.60	0.530 P	2.170 P	1.3500	0.153	x	0.1530
PI 468261	299	110.40	0.004 N	0.016 N	0.0100	0.098	0.099	0.0980
	24	54.15	3.312 P	1.768 P	2.5400	0.104	0.104	0.1040
PI 471952	264	63.50	0.006 N	0.339 P	0.1725	0.095	0.09	0.0920
	265	63.00	1.954 P	1.302 P	1.6280	0.165	x	0.1650
	149	72.30	0.004 N	0.004 N	0.0040	0.092	0.102	0.0970
	75	68.50	0.659 P	0.129 P	0.7880	0.321	0.296	0.3080
PI 493582	3	49.80	0.016 N	0.004 N	0.0028	0.422	0.414	0.4180
	272	50.40	2.786 P	0.008 N	1.3970	2.528	2.558	2.5430
	294	36.70	0.008 N	0.022 N	0.0150	0.257	0.258	0.2570
	94	37.70	0.437 P	0.372 P	0.4045	1.79	x	1.7900
PI 493965	45	53.75	0.002 N	0.011 N	0.0065	0.129	0.126	0.1275
	54	56.20	0.367 P	0.172 P	0.2695	0.195	x	0.1950
PI 494504	16	45.80	0.023 N	0.005 N	0.0140	3.957	3.927	3.9420
	86	37.78	3.359 P	1.329 P	2.3440	1.611	x	1.6110
PI 497422	242	64.40	0.007 N	0.000 N	0.0035	0.84	0.797	0.8180
	26	60.30	2.569 P	0.003 N	1.2990	0.488	0.47	0.4790
PI 602067	127	92.60	0.013 N	0.006 N	0.0095	11.25	11.387	11.3180
	77	76.00	0.948 P	0.223 P	0.5850	0.141	x	0.1410
PI 628560	108	67.20	0.004 N	0.009 N	0.0065	0.12	0.116	0.1180
	254	43.70	3.440 P	1.804 P	2.6220	0.26	0.285	0.2720
	156	54.90	0.004 N	0.023 N	0.0135	0.143	0.141	0.1420
	29	56.75	3.265 P	0.801 P	2.0330	0.108	0.101	0.1040
Average (N)	59.92				0.0238			1.2650
Average (P)	51.82				1.5688			1.574

Any pairs (negative versus positive) were from the same replicate plot for comparison

Since, other abiotic (i.e., drought, heat) and biotic stresses (i.e., insect damage) can also trigger plants to synthesize and accumulate more resveratrol (Sobolev *et al*., 2007) the field morphological observation data were retraced and the photographs of the plant status from the field observation are shown in pairs in Fig. 2. The photos show that both plants No. 25 and 127 were extremely stressed (either by other diseases or heat and drought) compared to their counterparts (No. 113 and 77). Plants No. 16 and 86 from accession PI 494504 were also extremely stressed and their seeds also accumulated high amounts of resveratrol (3.942 and 1.611 μg g^{-1}). By comparison, although plants No. 244 and 4 from accession PI 331314 were positive for TSWV, their stress levels were observed to be low and the accumulation of resveratrol in their seeds was also low (0.114 and 0.316 μg g^{-1}). We conclude that the high stress observed in the above-ground parts of these plants may partly explain the high levels of resveratrol in their seeds. In Table 5, we also found that seeds from plants infected with TSWV contained higher amounts of resveratrol than the plants without TSWV infection. For example, in four pairs of plants from two accessions of PI 471952 and PI 493582, all the seeds from TSWV-positive plants contained a statistically significant ($p<0.05$) higher amount of resveratrol (0.165, 0.308, 2.543 and 1.790 μg g^{-1}) than the seeds from TSWV-negative plants (0.092, 0.097, 0.418 and 0.257 μg g^{-1}). Regression analysis was also conducted using data in Table 5 (ELISA values as variable and resveratrol content as dependent variable). The regression model F value (data not shown) was not significant. The regression results confirmed that resveratrol variation could not be explained only by ELISA values (i.e., TSWV infection).

DISCUSSION

Plant response (susceptible/positive or resistant/negative) to TSWV can be evaluated by field observation or lab ELISA test. It should be more reliable to determine the plant response to TSWV based on both field observation and lab test. If the filed observation is too early (i.e., TSWV symptoms not fully developed on plants), some plants could be scored as false negative. The lab ELISA test also has its limitation. Within the same accession, different plants could be classified as TSWV positive or negative base on ELISA value (>0.1 or <0.1). There are a few possible explanations for these inconsistencies. First of all, a plant that tested negative initially that was left in a field harboring TSWV nearby has a certain likelihood of subsequently becoming infected by thrips transmitting the virus from an infected plant to a healthy plant. Samples that were initially found to be positive and later tested negative could have occurred due to the lack of available tissue sample collected since the two collections for ELISA testing were collected at different developmental stages. Therefore, if a particular plant was found positive initially the plant had time to deteriorate due to the viral infection and thus, tissue to perform the second ELISA replication was limited. Another possible explanation may result from the delineation of 0.1 as the cutoff between positive and negative. For example, two ELISA values for the plant number 66 were 0.188 and 0.096, respectively. Based on the first value (0.188), this plant was classified as positive (susceptible to TSWV); and based on the second value (0.096), this plant was classified as negative (resistant to TSWV). Actually, the second value 0.096 was very close to the 0.1 positive cutoff. This is where the discrepancy came from for the accession within the same year. In comparison with the previous study (Wang *et al*., 2007), there were five accessions (PI 247372, PI 468261, PI 493582, PI 493965 and PI 602067) in common for this study. The responses to TSWV for these five accessions were scored very similar but not exactly the same from two years observation. For example, from two years observation both PI 247372 and PI 493965 were scored as Moderate Tolerance (MT), PI 468261 as Moderate Susceptible (MS), PI 602067 as susceptible (S) but PI 493582 was scored as moderate susceptible (0-3.0) in this study and susceptible (S) in the earlier study. This score difference for PI 493582 could come from the difference of accession-neighboring when planting in the field or the bias of leaf sampling for the ELISA test. To determine whether the accession is really susceptible or resistant, more plants within the accession need to be assayed for at least two years in different locations. Furthermore, the response to TSWV should be scaled from 0 (highly resistant) to 10 (highly susceptible) based on the ELISA value. Enzyme-linked immunosorbent assay has been recommended for virus detection, but it should be done in optimum conditions. The results from ELISA can also be confirmed by more sensitive methods such as PCR or real-time PCR.

Plant response to TSWV may affect plant accumulation of resveratrol, however, resveratrol synthesis to detectable accumulation is a long and complicated process. During the process, any biological challenges (such as virus infection) and environmental stress (heat or drought) can change the resveratrol amount synthesized and accumulated. In this study, TSWV infection was only examined in the leaves and resveratrol levels were only assayed from the seeds. There are many factors and developmental stages which

were not considered in present experiment design and future experiments may include testing different tissue (leaves, roots, or seeds) over a period of time to assess resveratrol accumulation. In addition, the extraction method also may affect the amount of resveratrol quantified. One of our aims for this study was to investigate whether there was an association between plant response to TSWV and seed accumulation of resveratrol in peanut. Based on our data, we can only conclude that stress caused by TWSV infection may cause resveratrol to accumulate in seeds, but there are also other factors such as abiotic stress (drought or heat) and other diseases that may play a significant role in stimulating resveratrol synthesis and accumulation in peanut.

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REFERENCES

Alper, C.M. and R.D. Mattes, 2003. Peanut consumption improves indices of cardiovascular disease risk in healthy adults. J. Am. Coll. Nutr., 22: 133-141.

Baur, J.A. and D.A. Sinclair, 2006. Therapeutic potential of resveratrol: The *in vivo* evidence. Nature Rev. Drug Discov., 5: 493-506.

Chiteka, Z.A., D.W. Gorbet, F.M. Shokes, T.A. Kucharek and D.A. Knauft, 1988. Components of resistance to late ieafspot in peanut. I. Levels and variability-implications for selection. Peanut Sci., 15: 25-30.

Culbreath, A.K., J.W. Todd and S.L. Brown, 2003. Epidemiology and management of tomato spotted wilt in peanut. Ann. Rev. Phytopathol., 41: 53-75.

Holbrook, C.C., P. Timper and A.K. Culbreath, 2003. Resistance to *Tomato spotted wilt virus* and root-knot nematode in peanut interspecific breeding lines. Crop Sci., 43: 1109-1113.

Jain, R.K., S.S. Pappu, H.R. Pappu, A.K. Culbreath and J.W. Todd, 1998. Molecular diagnosis of *Tomato spotted wilt tospovirus* infection of peanut and other field and greenhouse crops. Plant Dis., 82: 900-904.

Jang, M., L. Cai, G.O. Udeani, K.V. Slowing and C.F. Thomas *et al*., 1997. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science, 275: 218-220.

Jonnala, R.S., N.T. Dunford and K.E. Dashiell, 2006. Tocopherol, phytosterol and phospholipid compositions of new high oleic peanut cultivars. J. Food Compos. Anal., 19: 601-605.

Lyerly, J.H., H.T. Stalker, J.W. Moyer and K. Hoffan, 2002. Evaluation of *Arachis* species for resistance to *Tomato spotted wilt virus* . Peanut Sci., 29: 79-84.

Pancholy, S.K., A.S. Despande and S. Krall, 1978. Amino acids, oil and protein content of some selected peanut cultivars. Proc. Am. Peanut Res. Edu. Assoc., 10: 30-37.

Pinnow, D.L., J.H. Chalkley and J.W. Demski, 1990. A practical method for the detection of peanut strip virus in peanut seed. Georgia Agric. Exp. Stat. Res. Rep., 584: 1-12.

Ross, J.A. and C.M. Kasum, 2002. Dietary flavonoids: Bioavailability, metabolic effects and safety. Annu. Rev. Nutr., 22: 19-34.

Sanders, T.H., R.W. McMichael and K.W. Hendrix, 2000. Occurrence of resveratrol in edible peanuts. J. Agric. Food Chem., 48: 1243-1246.

Singh, B. and U. Singh, 1991. Peanut as a source of protein for human foods. Plant Foods Human Nutr., 41: 165-177.

Sobolev, V.S. and R.J. Cole, 1999. *trans*-Resveratrol content in commercial peanuts and peanut products. J. Agric. Food Chem., 47: 1435-1439.

Sobolev, V.S., B. Guo, C.C. Holbrook and R.E. Lynch, 2007. Interrelationship of phytoalexin production and disease resistance in selected peanut genotypes. J. Agric. Food Chem., 55: 2195-2200.

Wang, M.L., D.L. Pinnow and R.N. Pittman, 2007. Preliminary screening of peanut germplasm in the US collection for TSWV resistance and high flavonoid content. Plant Pathol. J., 6: 219-226.

Wang, M.L. and R.N. Pittman, 2008. Resveratrol content in seeds of peanut germplasm quantified by HPLC. Plant Genet. Resour., 7: 80-83.

Wang, M.L., A.G. Gillaspie, J.B. Morris, R.N. Pittman, J. Davis and G.A. Pederson, 2008. Flavonoid content in different legume germplasm seeds quantified by HPLC. Plant Genet. Resour., 6: 62-69.