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Short Communication

Resveratrol content in seeds of peanut germplasm quantified by HPLC

M. L. Wang* and R. N. Pittman
USDA-ARS, PGRCU, 1109 Experiment Street, Griffin, GA 30223, USA

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Abstract

trans-Resveratrol (*trans*-3,4′,5-trihydroxystilbene), a polyphenolic compound uniquely identified in plants, is believed to greatly contribute to human health. Peanut (*Arachis hypogaea* L.) seeds of 20 germplasm accessions were harvested from the same field and used for resveratrol analysis by high-performance liquid chromatography. *trans*-Resveratrol content in air-dried peanut seeds was on average about 0.5 μg/g and a statistically significant variation (from 0.125 to 1.626 μg/g, at least a ten-fold difference) was detected among the accessions analysed. The average weight for 100 seeds was 52.84 g. A statistically significant variation in seed weight (from 22.30 to 87.94 g, at least a four-fold difference) was observed. There was no significant correlation between *trans*-resveratrol content and seed weight. The information about the levels of *trans*-resveratrol in peanut seeds will be useful for peanut cultivar development and peanut product processing. Breeders could use germplasm accessions containing a high amount of resveratrol to develop new nutritionally improved peanut cultivars and food processors could use these new cultivars to manufacture high resveratrol peanut products.

Keywords: *Arachis hypogaea* L; germplasm; high-performance liquid chromatography; *trans*-resveratrol content

Experimental and discussion

Peanut or groundnut (*Arachis hypogaea* L.) is an allotetraploid species ($2n = 4x = 40$) and one of the five most important oilseed crops in the world with a production of about 32 million metric tons of seeds (http://www.usda.gov/nass/pubs/agstas.htm). Peanut seeds contain 44–56% oil and 22–30% protein (Pancholy and Despande, 1978), and are therefore mainly used for edible oil production and as a high-protein food for human consumption, especially in developing countries where people have limited access to protein sources. Peanut seeds also contain useful phytochemicals, such as *trans*-resveratrol, which can contribute to human health (Jang *et al*., 1997). *trans*-Resveratrol (3,4′,5-trihydroxystilbene) is a polyphenolic compound initially classified as a phytoalexin (Ingham, 1976) due to its antifungal activity. *trans*-Resveratrol has recently drawn a great amount of attention from nutraceutical and pharmaceutical companies due to its antioxidant, anti-inflammatory and anti cancer activities, as well as chemopreventive, cardioprotective and estrogenic effects (Baur and Sinclair, 2006). Although resveratrol has been identified in peanut seeds, variation in resveratrol content of peanut germplasm accessions was unknown. The objectives of this study were to (1) determine the concentration and variability of resveratrol in peanut accessions of the USDA germplasm collection by high-performance liquid chromatography (HPLC) and (2) detect whether there is a

* Corresponding author. E-mail: mingli.wang@ars.usda.gov

correlation between resveratrol content and seed weight in peanut.

Twenty accessions were selected from the USDA peanut germplasm collection (http://www.ars-grin.gov/npgs/) and their botanical variety type and collection site are listed in Table 1. Seeds from each accession were planted in a 10-foot-long plot at Byron Georgia in 2007. There were no major diseases observed in the field. Matured pods were harvested, air-dried, shelled by hand and then the seeds were stored at 4°C until analysis. Dried seeds were counted, weighed and recorded in two replicates. A standard curve was generated with a *trans*-resveratrol reference purchased from Sigma (St Louis, MO, USA). Under bright light, *trans*-resveratrol could easily convert to *cis*-resveratrol. *trans*-Resveratrol was extracted by following the procedures described by Sanders *et al.* (2000), which were performed under yellow light. Approximately, 8 g of air-dried seeds were ground to a fine powder in a coffee blender. Ground seed tissues (3 g) were transferred into 50 ml Falcon tubes and homogenized with 9 ml of 80% ethanol using a Power Gen 125 homogenizer (Fisher Scientific, Loughborough, UK). The homogenized samples were centrifuged (Eppendorf, 5415D, Hamburg, Germany) at 12,000 ***g*** for 3 min. Two millilitres of the supernatant were taken and cleaned by solid-phase extraction using Poly-Prep chromatography column (0.8 cm × 4 cm, Bio-Rad) packed with ~1 ml mixture (1:1 w/w) of Al_2O_3 (EM Industries Inc., Gibbstown, NJ, USA) and silica gel 60 RP-18 (EMD Chemicals Inc., Hawthorne, NY, USA). 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 50°C 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, USA) at 40°C 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 with the following gradient: 10% B for 2 min, 10–30% B for 8 min, 30% B for 1 min, followed by 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 at 310 nm absorbance. *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 replicates were conducted for determination

Table 1. Selected accessions, seed weight and resveratrol content

Accession	Species	Identifier	Seed weight (g)*	Resveratrol (μg/g)†	Origin/collector
PI 520600	*Arachis hypogaea* L. var. *hypogaea*	TAMRUN 88	53.00 d	1.626 a	USA
PI 468261	*A. hypogaea* L. var. *hypogaea*	US 66	67.79 b	1.088 ab	Bolivia
PI 632380	*A. hypogaea* L. var. *hypogaea*	Georgia-02C	60.81 c	1.058 abc	USA
PI 590483	*A. hypogaea* L. var. *hypogaea*	US 1393	87.94 a	0.735 bcd	Brazil
PI 587093	*A. hypogaea* L. var. *hypogaea*	Georgia Green	50.28 def	0.732 bcd	USA
PI 149641	*A. hypogaea* L. var. *peruviana*	V-52	38.06 j	0.663 bcd	Brazil
PI 280688	*A. hypogaea* L. var. *hirsuta* J.	Guanajuato-2	42.07 hi	0.612 bcd	Mexico
PI 576638	*A. hypogaea* L. var. *hirsuta* J.	SSD 6	47.16 fg	0.489 bcd	Mexico
PI 531499	*A. hypogaea* L. var. *hypogaea*	OKRUN	53.06 d	0.389 bcd	USA
PI 497634	*A. hypogaea* L. var. *aequatoriana*	US 708	39.48 ij	0.338 bcd	Ecuador
PI 607535	*A. hypogaea* L. var. *hypogaea*	Florida MDR 98	67.78 b	0.319 bcd	USA
PI 502045	*A. hypogaea* L. var. *peruviana*	SPZ 470-2	51.47 de	0.308 bcd	Peru
PI 613135	*A. hypogaea* L. var. *hypogaea*	C-99R	63.24 c	0.308 bcd	USA
PI 259576	*A. hypogaea* L. var. *fastigiata*	Negro granda	41.75 hi	0.295 bcd	Paraguay
PI 602356	*A. hypogaea* L. var. *hirsuta* J.	WWT-1318	22.3 k	0.248 cd	Ecuador
PI 629027	*A. hypogaea* L. var. *hypogaea*	Georgia-01R	60.25 c	0.215 d	USA
PI 565448	*A. hypogaea* L. var. *hypogaea*	FLORUNNER	49.84 def	0.207 d	USA
PI 506419	*A. hypogaea* L. var. *hypogaea*	Southern Runner	47.98 ef	0.181 d	USA
PI 501297	*A. hypogaea* L. var. *hirsuta* J.	US 1260	68.67 b	0.177 d	Peru
PI 502043	*A. hypogaea* L. var. *peruviana*	SPZ 471-1	43.85 gh	0.125 d	Peru
Mean			52.84	0.503	
LSD			3.593	0.817	

* Gram per 100 seeds; † a–d: if the letters are the same after values, there is no significant difference.

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of *trans*-resveratrol concentration. For each replicate, two sample extractions were conducted after grinding for data collection. The average from two extractions per sample was used for data analysis. One-way analysis of variance was conducted using statistical analysis system (SAS OnlineDoc® 9.1.3, 2004) to analyse the data, and Fisher's protected least significant difference (LSD) test was used to separate means. Pearson's correlation coefficient analysis was performed to determine the interrelationship between resveratrol concentration and seed weight.

As an example, compiled chromatograms for detecting the amounts of *trans*-resveratrol from three peanut varieties using HPLC are shown in Fig. 1. The arrow-marked peak areas correspond to the amounts of *trans*-resveratrol detected. The accessions of PI 502043, PI 149641 and PI 520600 produced peaks with a small, medium and large area, respectively. In these single extractions, 0.09, 0.70 and 1.46 μg/g of *trans*-resveratrol, respectively, were detected for these three accessions (Fig. 1). Variation for *trans*-resveratrol from peanut accessions is statistically significant at $P < 0.0001$. The variation from replicates was not statistically significant at $P < 0.05$. Therefore, the variability of *trans*-resveratrol content detected by HPLC mainly came from differences in the peanut varieties. *trans*-Resveratrol contents in peanut seeds are summarized in Table 1. Among 20 accessions quantified, the seeds of PI 520600 (TAMRUN 88, a cultivar developed in Texas, USA) contained a significantly higher amount of *trans*-resveratrol (1.626 μg/g labelled as 'a' in Table 1) than the other 19 accessions. The seeds from PI 501297, PI 502043, PI 506419, PI 565448 and PI 629027 contained a significantly lower amount of *trans*-resveratrol (0.177, 0.125, 0.181, 0.207 and 0.215 μg/g labelled as 'd' in Table 1) than the accessions of PI 428261, PI 520600 and PI 632380 (1.088, 1.626 and 1.058 μg/g labelled as 'ab, a and abc', respectively, in Table 1). All three accessions (PI 428261, PI 520600 and PI 632380) that contained a high amount of *trans*-resveratrol in the seeds were from *A. hypogaea* L. var. *hypogaea*. The amount of *trans*-resveratrol in the seeds of Florunner, a released cultivar developed in Florida, USA, had been quantified in this and other studies (Sobolev and Cole, 1999). The results from these two separate studies are comparable (0.207 μg/g from this study and 0.273 μg/g averaged from Sobolev and Cole's study).

The weights for 100 seeds of the analysed accessions are listed in Table 1. A statistically significant variation

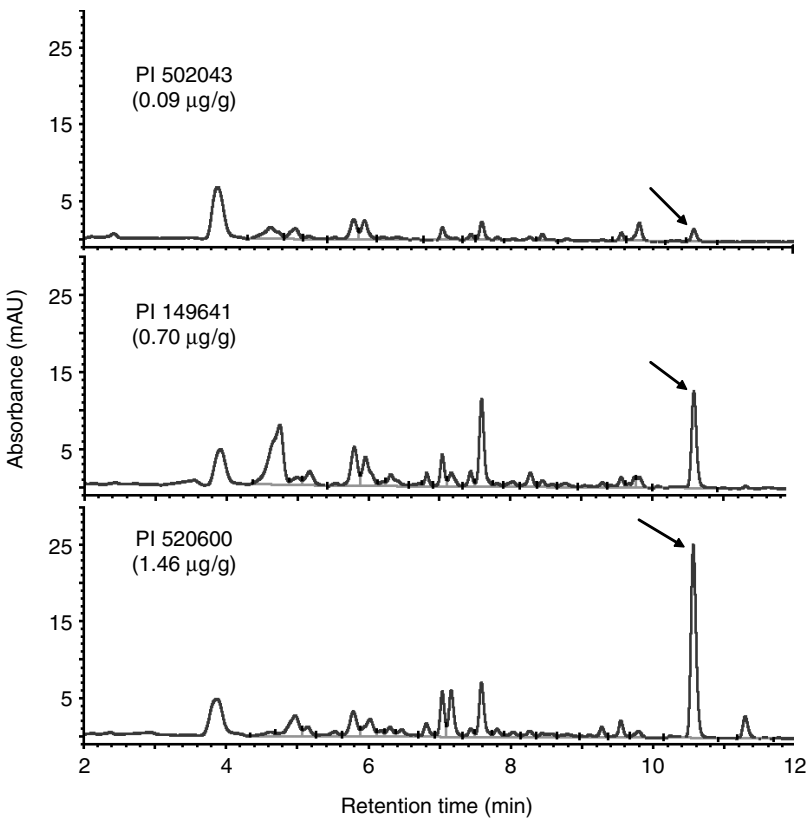

Fig. 1. Metabolite chromatograms from the peanut extracts analysed by HPLC. Arrows indicate *trans*-resveratrol peaks in the analysed extracts.

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in 100 seed weights was observed at $P < 0.0001$. The average weight for 100 seeds was 52.84 g, ranging from 22.30 to 87.94 g (at least a four times difference). From our study, there was no significant correlation between *trans*-resveratrol content and seed weight observed. The results from a previous report (Sobolev and Cole, 1999) showed decreasing *trans*-resveratrol with increasing seed size. Seed weight and size are not interchangeable, but a larger seed usually has a higher seed weight. The trend for decreasing *trans*-resveratrol with increasing seed size was not observed in the correlation between resveratrol content and seed weight. The inconsistent results from these two studies may be explained by the following reason. Seed weights in our study were from different accessions and reflected the difference from genotypes, whereas seed sizes in their study were from one accession (Florunner) and mainly reflected the difference in seed maturity (i.e. mature seeds had a larger size whereas immature seeds had a smaller size).

Significant variability was observed in the amounts of resveratrol among seeds of 20 peanut accessions. There is a potential to increase the *trans*-resveratrol amount in peanut cultivars. *trans*-Resveratrol could be added as a selection trait in breeding programmes to develop high resveratrol peanut cultivars. Furthermore, high resveratrol peanuts should be considered for use in peanut product processing. Since consumption of peanuts and/or peanut products containing a high amount of resveratrol can contribute to human health, developing high resveratrol peanut cultivars may eventually benefit peanut farmers and processors as well as consumers.

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