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Peanut antioxidants: Part 1. Genotypic variation and genotype-by-environment interaction in antioxidant capacity of raw kernels

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ABSTRACT

The potential to select for antioxidant traits in peanut (*Arachis hypogaea* L.) kernels by conventional plant breeding was investigated by oxygen radical absorbance capacity (ORAC) assay of (a) 32 full-season maturity genotypes grown at Kairi Research Station in 2008/09; and (b) a subset of ten genotypes with diverse antioxidant capacities grown in five different production regions in Queensland in 2009/10 and 2010/11. There were significant levels of variation of more than 25% relative standard deviation (RSD) in antioxidant capacity among the 32 tested genotypes; and that genotype, environment, and genotype-by-environment (G × E) interaction all significantly ($P < 0.05$) affected trait expression. Analysis of the G × E data confirmed the heritability of antioxidant capacity, which has not been previously quantified, whereby genotype explained 44% of the phenotypic variation on a plot basis and 82% on an entry mean basis. This indicated that there was substantial genetic control of antioxidant capacity in peanut kernels, but also that it will be important to characterize environmental interaction to enable plant/seed selection in the Breeding Program. The research also highlighted the utility of the ORAC assay for screening germplasm and provided practical advice on design of varietal assessments using antioxidant screenings.

1. Introduction

The Australian peanut industry faces several major challenges including difficulties in maintaining productivity and continuity of supply in the face of cost/price pressures and extreme seasonal fluctuations; competition with cheaper imported products from countries with lower costs of labor and production; and stringent demands to ensure food safety and quality (Mills et al., 2001). Like most Australian agri-food industries, peanut producers and manufacturers rely on the production of high-quality rather than bulk-quantity products to compete in the global marketplace. The development of peanut products with enhanced quality attributes would benefit the industry with opportunities for value-adding and niche-marketing, as well as benefit consumers and contribute to improved public health through increased access to healthier snack food options that have more nutritional 'bang-for-the-buck'.

The antioxidant properties of food and beverage products have attracted intense research and commercial interest due to the widely-accepted importance of dietary antioxidants in the cellular defense repertoire against oxidative tissue injury, alongside complex enzymatic defense mechanisms (Castro & Freeman, 2001). Dietary antioxidants include both hydrophilic (e.g., ascorbate and polyphenols) and lipophilic (e.g., tocopherols, flavonoids, and carotenoids) radical scavengers that counteract the initiation and propagation of oxidative injury to DNA, lipids, and proteins by reactive oxygen and nitrogen species. Such oxidative stress is implicated in the sustained inflammation, cell proliferation, cytotoxicity, and pro-angiogenic environment common to the pathophysiology of diverse diseases (Castro & Freeman, 2001) including carcinoma and cardiovascular disease, the classic oxidative stress-related diseases. There is also rapidly expanding data linking oxidative injury to conditions as varied as neurodegenerative, renal, and liver diseases, diabetes, atherosclerosis, fetal vascular syndrome in pre-eclampsia, metabolic syndrome, and ocular degeneration (Roberts et al., 2009; Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005).

The protective or therapeutic effects of dietary antioxidant intake against chronic disease have been suggested most

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convincingly by epidemiologic studies of dietary patterns and health; however clinical trials of antioxidant interventions (such as vitamin supplements) have yielded inconsistent results (Arts & Hollman, 2005; Bhupathiraju & Tucker, 2011). Nevertheless, the health benefits of regular peanut consumption are at least partially attributed to enhanced intake of dietary antioxidants and contingent protection against oxidative damage, especially reduced risks of developing cancer, cardiovascular disease, and Alzheimer's disease (Isanga & Zhang, 2007).

Plant breeding to improve the antioxidant profile of peanut kernels presents a novel approach to the eventual development of peanut products with enhanced 'functional food' quality. Genetic improvement is a key strategy by which agricultural industries improve productivity and meet market demands, but application to nutritional quality and health-related traits is new in breeding programs (Christinck & Weltzien, 2013). Peanuts are bred conventionally in Australia, so it is crucial to establish the stability and levels of naturally-occurring genotypic variation among the germplasm collection in order to ascertain the potential to select for higher levels of the trait, which has not been previously quantified. Numerous methods have been proposed for high-throughput *in vitro* assessment of 'total antioxidant capacity', which have been reviewed extensively, most recently by Gülçin (2012) and Karadag, Ozcelik, and Saner (2009). Such *in vitro* assays have frequently been used for measurement of composite or retail samples, but rarely applied to comparison of genotypes.

This paper describes studies of the genotypic variation affecting kernel antioxidant capacity and the importance of genotype-by-environment (G × E) interaction using the ORAC assay for high-throughput screening of Australian peanut germplasm. The primary objectives of the research were to evaluate the breeding potential of antioxidant-related traits in peanuts, and to identify a range of high- and low-antioxidant genotypes for chromatographic profiling and other subsequent research.

2. Materials and methods

2.1. Instruments

A PolarSTAR Optima multi-mode microplate reader (BMG Labtech GmbH, Ortenberg, Germany) installed with 485 nm and 520 nm filters and two onboard reagent injectors was used to measure fluorescence intensity in the ORAC assay, which was performed in black 96-well FLUOTRAC 200 (flat bottom, chimney well, medium-binding) microplates (Greiner Bio-One GmbH, Germany).

2.2. Reagents

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), fluorescein, and 2,2′-azobis (2-amidino-propane) dihydrochloride (AAPH) were purchased from Sigma Inc. (St. Louis, MO, USA). Trolox and fluorescein stock solutions were frozen in aliquots until use, while AAPH was made fresh at the time of analysis. Randomly methylated β-cyclodextrin (RMCD) was obtained from Cyclodextrin Technologies Development Inc. (High Springs, FL, USA). The extraction solvents were analytical grade. All assay solutions were made up in phosphate buffer (75 mol m^{-3}, pH 7.4).

2.3. Samples

Samples in the genotype study comprised 32 full-season maturity peanut genotypes (140 days; *Arachis hypogaea* ssp. *hypogaea*) grown in 2008/09 under the auspices of the Australian Peanut Breeding Program, which is collaboratively run by the Peanut Company of Australia, Grains Research and Development Corporation, and Queensland Department of Agriculture, Fisheries and Forestry. The genotypes represented current commercial cultivars in addition to new breeding lines progressing to prospective release.

A subset of ten full-season genotypes representing a large range of antioxidant capacity was selected for the study of G × E interaction. In addition, six ultra-early maturity genotypes (100–110 days; *A. hypogaea* ssp. *fastigiata*) were also evaluated. The samples were harvested from five distinct growing environments in the principal peanut production regions of Australia in 2009/10 and 2010/11. The sites were Bundaberg Research Station (RS) and a commercial farm (Bundaberg Russo) in the coastal Burnett region of Queensland, Redvale RS and Taabinga RS near Kingaroy in the south Burnett, and Kairi RS in the Atherton Tablelands of northern Queensland.

The peanut plants were grown in triplicate randomly-allocated field plots under non-limiting (nutrient- and water-replete) conditions. Samples (200 g) of sound, mature, jumbo and size 1 kernels (>11.9 mm kernel diameter for Virginia market type, >9.9 mm for Runner, and >9.5 mm for ultra-early) were de-hulled, dried to approximately 7% moisture content in accordance with commercial practice, transported to the University of New South Wales, and stored in a cool room (3–5 °C) in vacuum-sealed polyethylene bags until the time of analysis.

2.4. Preparation of crude extracts

Crude extracts were prepared according to the generic method developed by Prior et al. (2003). Peanut kernels (10 g) were embrittled in liquid nitrogen then pulverized in an electric coffee grinder after manual removal of the testa. Ground sample (1.0 g) was vortex-mixed with *n*-hexane (10 mL), sonicated (50 kHz, 37 °C, 5 min), and centrifuged (1620 *g*, 15 min). The supernatant was collected and the extraction repeated. The combined supernatant was evaporated by flushing with nitrogen gas, and re-solubilized in acetone (5.0 mL) and 1% (w/v) RMCD/methanol (5.0 mL) to produce the lipophilic extract. The peanut residue was flushed with nitrogen gas to evaporate any residual *n*-hexane and then extracted by sonication (50 kHz, 37 °C, 5 min) in 70:29.5:0.5 (v/v/v) acetone/water/acetic acid (10.0 mL). The tube was centrifuged (1620 *g*, 15 min) and the supernatant used as the hydrophilic extract.

2.5. Moisture content

The moisture content of the peanut samples was estimated gravimetrically (Young et al., 1982) and used to convert the values of antioxidant capacity to a dry weight basis. Ground sample (2 g) was accurately weighed in an oven-dried aluminum pan and heated in a convection oven (130 °C) for 2 h (i.e., until constant weight according to a preliminary experiment). The dish was reweighed after cooling in a desiccator, and the weight loss considered to represent moisture content.

2.6. ORAC assay

The ORAC assay described by Prior et al. (2003) was optimized for our sample type and laboratory conditions. The reaction mixture comprised the standard or sample extract (25 μL), fluorescein (150 μL, 70 μmol m^{-3} for hydrophilic assay or 35 μmol m^{-3} for lipophilic), and AAPH (25 μL, 140 mol m^{-3}). Standards and extracts were manually transferred to the microplate using a single-channel pipette, while the fluorescein and AAPH were automatically delivered by the microplate reader. The fluorescein and AAPH were pre-incubated at 37 °C for 60 and 30 min prior to use. The microplate reader was operated at 37 °C, and fluorescence (λ_{ex}