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Breeding for improved blanchability in peanut: phenotyping, genotype × environment interaction and selection

G. C. Wright[A,B,F], *M. G. Borgognone*[C], *D. J. O Connor*[A,B], *R. C. N. Rachaputi*[B], *R. J. Henry*[B], *A. Furtado*[B], *N. L. Anglin*[D], *and D. B. Freischfresser*[E]

[A]Peanut Company Australia, Kingaroy, Qld 4610, Australia.
[B]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, Qld 4072, Australia.
[C]Crop and Food Science, Agri-Science Queensland, Department of Agriculture and Fisheries, Toowoomba, Qld 4350, Australia.
[D]USDA ARS Plant Genetic Resources Conservation Unit, Griffin, GA 30223, USA.
[E]AgriSciences Queensland, Department of Agriculture and Fisheries, Kingaroy, Qld 4610, Australia.
[F]Corresponding author. Email: graeme.wright@bega.com.au

Abstract. Breeding for improved blanchability—the propensity of the testa (skin) to be removed from the kernel following rapid heat treatment—is a priority for improvement in the Australian Peanut Breeding Program (APBP). Easy removal of the testa by blanching is required for processing of peanuts into peanut butter and various other confectionary products. Thus, blanchability is an economically important trait in any newly released cultivar in Australia. A better understanding of the range of genetic variation, nature of inheritance and genotype × environment (G×E) interactions, and the development of a low-cost method to phenotype in early generations, could speed up breeding for this trait. Studies were conducted to develop a low-cost, rapid method utilising minimal amounts of seed to phenotype in early generations, along with an assessment of G×E interactions over a range of years and environments to derive optimal selection protocols. Use of a smaller kernel sample size than standard (50 vs 200 g) was effective for accurately assessing blanchability in breeding lines and could allow selection in early generations (e.g. in seed produced from a single F_2 plant where seed supply is adequate). G×E interaction for blanchability was shown to be very low. Genotypic variance explained 62–100% of the total variance for blanchability, assessed in two diverse germplasm pools including 107 accessions in the USA mini-core over three environments and multiple APBP breeding lines grown over nine different years–environments. Genotypic correlations between all environments were very high (~0.60–0.96), with heritability for the blanchability trait estimated to be very high (0.74–0.97) across the 13 trials. The results clearly demonstrate that effective selection for improved blanchability can be conducted in early generations and in a limited number of contrasting environments to ensure consistency of results.

Additional keywords: *Arachis hypogaea*, G × E, groundnut, quality.

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Introduction

Blanching, the complete removal of the testa (skin) from the kernel by heating followed by abrasion, is a highly desirable quality trait in peanut (*Arachis hypogaea* L.). Shellers and blanchers normally blanch a very high proportion of their peanut intake (>80%) before on-selling to peanut processors for the manufacture of a range of confectionary products including peanut butter, snack food, snack bars, peanut flour and others (Singh *et al*. 1996; Sanders *et al*. 1999). The costs associated with blanching are significant, estimated to be equivalent to the respective costs of shelling and crop production (R. B. Hansen, Peanut Company of Australia (PCA), pers. comm., 2009). This value-adding process subjects the peanut kernels to additional cleaning and sorting, which assists in aflatoxin reduction via the efficient removal of damaged and discoloured kernels by colour sorting (Whitaker *et al*. 2005). Blanchability is strongly affected by genotype (Singh *et al*. 1996; Cruickshank *et al*. 2003; Janila *et al*. 2012; Wright *et al*. 2013) and by maturity and harvest date (Farouk *et al*. 1977; Mozingo 1979); hence, any genotypic or environmental effect that reduces kernel blanchability can greatly increase processing costs.

Considering the strong genotypic influence, it is surprising that selection for good blanchability is largely neglected in most global peanut-breeding programs, especially in the USA, given its economic importance in peanut butter and edible

kernel processing. By contrast, in Australia, blanching has been a key selection trait in the Australian Peanut Breeding Program (APBP) since the mid-1990s, after a popular drought-tolerant variety, Streeton, was rejected by PCA for its poor blanching characteristics (Cruickshank *et al*. 2003). The APBP subsequently developed a laboratory-scale blancher, based on a modified Ashton abrasive-roller blanching unit, to screen peanut genotypes effectively for blanchability. This blancher was also shown to be an excellent predictor of commercial blanching performance (Cruickshank *et al*. 2003), unlike an earlier laboratory-scale blanching unit (Wright and Mozingo 1975). In the APBP, any new variety being considered for commercial release must have a blanchability score >85%, which is generally considered the industry standard for acceptable blanchability (Cruickshank *et al*. 2003; Schirack *et al*. 2007).

A few studies have indicated that blanchability is under strong genetic control, is highly heritable and should be amenable to effective selection in breeding programs (Shokraii *et al*. 1985; Cruickshank *et al*. 2003). Indeed, Cruickshank *et al*. (2003) concluded that parental selection for good blanchability was the most important factor in breeding for this trait. However, published data on the extent of genetic variability for the blanching trait are scarce in a more diverse collection of cultivated peanut germplasm. Additionally, limited information is available about the stability of the blanchability trait across different environments; hence, the extent of genotype × environment (G×E) interaction, which is important to the efficiency of breeding for this trait, is currently lacking.

Furthermore, although early-generation selection is theoretically possible and actually desirable in a breeding program, Cruickshank *et al*. (2003) suggested that a significant quantity of seed was required to phenotype single plants accurately (e.g. >200 g kernels). In practice, this has been very difficult to achieve because most single plants produce only ~50–300 g, and most of this seed is required to progress the line for multiple-trait assessment in the next generation. As such, phenotyping and selection for blanchability have been conducted only in later generations when seed quantities are more plentiful, which means that a breeder may have to wait multiple generations to determine whether a new line meets the standard of >85% blanchability. However, if blanchability could be accurately assessed on a smaller quantity of seed (e.g. 50 g), it should be possible to phenotype individual plants/lines effectively and hence make early-generation selection decisions at the F_2 or F_3 stage.

The objectives of this study were as follows: (*i*) to develop a phenotyping protocol that could be used to evaluate blanchability from seed derived from a single plant produced in early generations after crossing; (*ii*) to evaluate the extent of genetic diversity, genotypic variability and G×E interaction; and (*iii*) to estimate heritability for blanchability across wide germplasm pools in order to develop efficient selection and breeding guidelines for this trait.

Materials and methods

Standard procedure for blanching peanut samples

Kernel size can influence blanchability (Mozingo 1979; Shokraii *et al*. 1985); therefore, all blanching samples comprised only mature kernels (Jumbo and 1 size) and splits graded by using standard peanut grading screens appropriate to each genotype. For the Virginia market types, which are typically the larger seed of all peanut market types, this meant using kernels passing over a 10.7-mm-diameter holed screen. Whereas for the Runner and Spanish market types, a 9.1-mm holed screen was used. This grading was conducted to eliminate kernel size and maturity as possible sources of variation in blanchability.

The initial weight of each sample (pre-blanching weight) for all experiments was recorded, with initial target weights for samples varying depending on the experiment. Blanching was initiated by heating kernel samples, individually placed in trays, for 1 h in fan-forced ovens that had been pre-heated at 95°C, followed by turning the ovens off, which resulted in kernels cooling to room temperature over the next 8 h.

After cooling, samples were passed over a single-unit Ashton whole-nut abrasive-roller blancher (Ashton Food Machinery, Newark, NJ, USA) for varying periods, depending on the experiment. This blanching device was known to be effective in discriminating between genotypes with good and poor blanching characteristics, and to produce results highly correlated with commercial-scale blanching results (Cruickshank *et al*. 2003).

Whole kernels and splits were considered blanched when <3 mm^2 testa remained on each kernel or split. The combined weight of blanched kernels and splits was recorded (blanched weight) for each sample by sorting the seeds post-processing into blanched and unblanched kernels. Blanching percentage was calculated as 100 × blanched weight/pre-blanching weight.

Development of a new phenotyping protocol for blanchability for limited sets of seed

In order to obtain a protocol that mirrors commercial blanching processing, two experiments were conducted to explore the effects on blanching of genotype and sample size (Expt 1), and genotype, sample size and abrasion time (Expt 2) (Table 1). The existing standard blanching protocol in the APBP involves a 200-g sample and a 20-s exposure to abrasion in the blancher, which is often not possible to

Table 1. Experimental details for the phenotyping protocol study

Factor	Factor level
Experiment 1: 16 treatments (4 genotypes × 4 sample sizes)	
Genotype (blanchability)	Holt (good), Middleton (good), D48-4-p4-1 (average), P13-p07-219 (poor)
Sample size (g)	20, 50, 100, 200
Abrasion time (s)	20
Replicates	4
No. of ovens	2
Experiment 2: 16 treatments (2 genotypes × 2 sample sizes × 4 abrasion times)	
Genotype (blanchability)	Holt (good), P13-p07-218 (poor)
Sample size (g)	50, 200
Abrasion time (s)	5, 10, 20, 40
Replicates	4
No. of ovens	2

achieve because of limited seed-set in early generations of the breeding cycle. Therefore, various genotypes, sample sizes and abrasion times were tested to develop a new protocol using less seed.

Raw kernels of two commercial varieties and two breeding lines (Holt, Middleton, D48-4-p4-1, and closely related sister lines P13-p07-219 or P13-p07-218) were sourced from the APBP in Kingaroy, Queensland (26°33′S, 150°50′E). Holt and Middleton are considered good blanchers (>85–90% blanching), D48-4-p4-1 is considered an average blancher (70–85% blanching), and P13-p07-219 and P13-p07-218 are poor blanchers (<70% blanching).

Experiment 1 examined 16 treatments in a factorial arrangement of two factors, genotype and sample size, with four levels each (Table 1), and all samples receiving a 20-s abrasion time in the blancher. Experiment 2 examined 16 treatments in a factorial arrangement of three factors, genotype and sample size with two levels each, and abrasion time with four levels (Table 1). Both experiments included had four replicates (Table 1).

The two ovens used for the experiments contained eight racks, each rack accommodating four treatments in a two-row by two-column arrangement. Racks 1–4 and racks 5–8 accommodated complete replicates of the 16 treatments in each oven. Treatments were randomised within each replicate, ensuring that they were balanced in their allocation to racks and positions within racks across replicates. Samples were processed in the blancher in rack order within the oven.

Blanching percentage for each experiment was analysed by using the analysis of variance (ANOVA) procedure in Genstat 16th Edition (VSN International, Hemel Hempstead, UK). Models for the two experiments included blocking terms for oven and replicate within oven, terms for interactions between the factors included in each experiment, and a random error term. Multiple comparisons for the treatment means associated with the significant terms in the ANOVA were performed by using the least significant difference (l.s.d.) test with the significance level set at $P = 0.05$. Examination of the residual plots from the ANOVA for each experiment did not indicate severe deviations from the assumptions of normality and homogeneity of variance. These experiments demonstrated that the optimum combination was 50-g samples with a 10-s abrasion time, and this was selected as the standard blanching protocol for all remaining experiments.

Genotypic variability in Australian and US genotypes, G×E interaction, and heritability for blanchability

Three series of multi-location–year field experiments were studied that included a wide range of peanut germplasm. The pods from each plot in the field were dried to 10% moisture (standard in the Australian peanut industry) after harvesting, and samples were stored at room temperature for up to a month until shelling. After shelling, kernels were graded and samples of graded kernels with a moisture content of 6% (± 1%) from each plot in the field were stored in plastic, zip-lock bags at 6°C until the blanching procedure was performed, usually within 3–4 weeks of shelling.

Phenotyping the US mini core (USMC) germplasm series in two environments in Australia and the USA (2013, 2014)

The first series included three field experiments established to assess variation of blanchability in accessions from the USMC collection (Holbrook and Dong 2005). Two of these experiments were at the Queensland Department of Agriculture and Fisheries (QDAF) Taabinga Research Station (26.5°32′S, 151°50′E), Kingaroy, Queensland, during the 2012–13 and 2013–14 summer seasons (AU13 and AU14, respectively). The third experiment (US13) was at the University of Florida Citra Field Station (29°24′N, 82°06′W) in Citra, Florida, USA, during the 2013 summer season.

The AU13 and AU14 trials were planted in single-row plots 5 m long with a 0.9-m inter-row spacing and seeding rate of 10 plants m^{-1}. Trials were fully irrigated to prevent crop-water deficits, with crop management according to best practice for soil nutrition, and pest and disease control. Four replicates of 73 accessions were planted in AU13 and three replicates of 98 accessions in AU14, according to Latinised row–column designs. These two trials had 72 accessions in common (Table 2); one accession (PI 196635) was not included in AU14 owing to lack of suitable seed for planting. Because these accessions from the USMC are mainly landraces rather than improved lines, several replicate plots had poor growth and seed-set and were not able to be harvested in these trials, meaning that kernels were not available for blanching. These were treated as missing plots.

The US13 trial was part of a wider phenotypic, biochemical and genotypic study to catalogue the entire US core collection comprehensively and was planted in two-row plots of length 10 feet (~3 m) with inter-row spacing 30 inch (~0.76 m) and seeding rate of 50 plants per row. A plot-skip of 10 feet (~3 m) was incorporated between all plots to avoid mixing among genotypes. Irrigation was applied to prevent crop-water deficits, and a full fungicide spray program was employed to control foliar diseases, which primarily included early and late leaf spot. Accessions were planted following an augmented design with three blocks. Accessions included three replicates of 107 USMC accessions (one in each block), one replicate of 687 accessions from the US core collection (randomly allocated to one of the three blocks), and either three or nine replicates of 14 commercial varieties including each market type (Runner, Virginia, Spanish and Valencia), depending on seed availability. Peanut Runner genotypes used included Florida 07 (PI 652938, Gorbet and Tillman 2009), Red River Runner (PI 665474, Melouk *et al*. 2012), FloRun 107 (PI 663993, Tillman and Gorbet 2015), GA06G (PI 644220, Branch 2007) and Tifguard (PI 651853, Holbrook *et al*. 2008). Spanish genotypes used included Tamnut OL 06 (PI 642850, Baring *et al*. 2006), Olin (PI 631176, Simpson *et al*. 2003) and Tamrun OL 11 (PI 665017, Baring *et al*. 2013). Virginia genotypes used included Bailey (PI 659502, Isleib *et al*. 2011), Jupiter (Anon. 2000), and Florida Fancy (PI 654368, Plant Variety Protected #200800231, Sept, 2012). Valencia genotypes used included NuMex 01 (PI 670460, Puppala and Tallury 2014), New Mexico Valencia A (PI

Table 2. US mini core (USMC) series trials evaluated in Australia (AU13, AU14) and the USA (US13)
Values are for commercial varieties and USMC accessions (PI numbers), obtained from the factor analytic (FA1) model fitted to the trials. Number of blanching replicates may not coincide with number of field replicates owing to seed availability. Entries are presented in descending overall blanching percent order; commercial varieties first, followed by PI accessions

Entry	AU13	AU14	US13	Overall predicted blanching (%)	Approximate standard error
	(no. of blanching replicates)				
Florida 07			3	93.1	4.57
Tamnut OL 06			9	92.0	2.85
Olin			9	91.5	3.05
Red River Runner			3	91.0	4.45
Bailey			9	90.7	3.05
FloRun 107			9	89.6	3.35
Tamrun OL 11			3	89.1	4.83
Florida Fancy			9	88.6	3.36
GA06G			9	85.7	3.68
NuMex 01			3	79.1	6.31
Jupiter			3	76.8	6.54
GenTex H & W 136			9	69.0	4.86
New Mexico Valencia A			9	65.8	5.00
Tifguard			3	53.4	7.74
PI 268696		3	3	94.4	2.04
PI 504614		3	3	92.8	2.30
PI 155107	4	3	3	91.1	1.77
PI 268806	4	3	3	91.1	1.78
PI 337399	4	3	3	90.7	1.81
PI 481795	2	3	3	90.6	1.91
PI 319768	4	3	3	90.4	1.84
PI 200441	4	3	3	90.0	1.87
PI 240560		3	3	89.8	2.69
PI 152146	4	3	3	89.1	1.94
PI 162655		3	3	88.9	2.79
PI 482120	4	1	3	88.8	2.26
PI 407667	4	2	3	88.2	2.11
PI 288210	4	3		88.2	1.70
PI 482189	4	3	3	88.2	2.11
PI 288146	4	2	3	88.0	2.13
PI 268755	4	3	3	87.7	2.05
PI 319770	3	3	3	87.7	2.09
PI 268586			3	87.4	5.16
PI 290560	4	2	3	87.2	2.19
PI 271019	4	3	3	86.9	2.10
PI 296550	4	3	3	86.9	2.11
PI 337293		3	3	86.0	3.09
PI 259658			3	85.6	5.45
PI 461434	4	3	3	85.6	2.19
PI 408743			3	84.9	5.56
PI 323268	4	3	3	84.1	2.28
PI 290594	4	3	3	83.2	2.33
PI 478819	4	3	3	83.2	2.33
PI 290620		3	3	82.9	3.35
PI 471954	4	3	3	82.6	2.36
PI 270907	4	3	3	82.2	2.38
PI 270786	4	3	3	82.1	2.39
PI 296558	4	3	3	82.0	2.40
PI 343398		3	3	82.0	3.42
PI 259851		3	3	81.6	3.44
PI 259836		3	3	80.7	3.51
PI 372305	4	3	3	80.6	2.47
PI 355271	4	3	3	79.3	2.53
PI 433347			3	78.4	6.39
PI 371521		3	3	77.9	3.69

(*continued next page*)

Table 2. (*continued*)

Entry	AU13	AU14	US13	Overall predicted blanching (%)	Approximate standard error
	(no. of blanching replicates)				
PI 274193		3	3	77.5	3.71
PI 476636		3	3	77.0	3.74
PI 497395			3	76.9	6.54
PI 429420	4	3	3	76.7	2.64
PI 162857	4	3	3	76.7	2.64
PI 493693	4	3	3	76.5	2.64
PI 157542	4	3	3	76.3	2.65
PI 338338	4	2	3	76.1	3.06
PI 355268	4	3	3	75.9	2.67
PI 493717		2	3	75.8	4.03
PI 290536	4	3	3	75.8	2.67
PI 494018	4	3	3	75.6	2.68
PI 270905		3	3	75.5	3.82
PI 290566	4	3	3	75.4	2.68
PI 497318	4	3	3	75.4	2.69
PI 494034		1	3	75.1	4.56
PI 442768	3	3	3	74.5	2.76
PI 268996	4	3	3	74.3	2.73
PI 478850	4	3	3	73.6	2.75
PI 295250	4	3	3	73.3	2.76
PI 493356		2	3	73.1	4.17
PI 493329	4	3	3	73.1	2.77
PI 493880	4	2	3	73.1	2.90
PI 476432			3	72.9	6.90
PI 270998	4	3	3	72.8	2.78
PI 295730	4	3	3	72.7	2.78
PI 159786			3	72.6	6.93
PI 461427	4	3	3	72.1	2.80
PI 494795	4	3	1	71.9	3.56
PI 196635	4		3	71.4	3.73
PI 403813		1	3	70.8	4.80
PI 475918	4	3	3	70.3	2.85
PI 471952	4	3	3	69.9	2.86
PI 372271		3	3	69.7	4.09
PI 502040	4	1	1	69.2	4.14
PI 331297	4	3	3	68.8	3.03
PI 356004	4	1	3	68.2	3.33
PI 196622	4	3	3	67.7	3.20
PI 313129		3	3	65.7	4.22
PI 268868	4	3	3	65.1	2.97
PI 493631	4	3	3	64.8	2.98
PI 502120	4	3	3	64.6	2.98
PI 259617	4	3	3	64.6	2.98
PI 475863	4	3	3	64.4	2.99
PI 295309	4	3	3	64.4	2.99
PI 158854	4	3	3	63.6	3.00
PI 298854	4	3	3	62.9	3.01
PI 497639	4	3	3	62.8	3.02
PI 292950			3	62.1	7.55
PI 331314		2	2	61.8	5.05
PI 399581	4	2	3	61.2	3.19
PI 337406		3	3	60.4	4.35
PI 493938	4	3	3	59.4	3.06
PI 370331		3	3	58.5	4.38
PI 343384		1	3	58.4	5.20
PI 325943	4	3	3	56.6	3.09
PI 262038	4	3	3	55.9	3.09
PI 493581		3	3	51.9	4.44
PI 493729	4	3	3	51.7	3.11

(*continued next page*)

Table 2. (*continued*)

Entry	AU13	AU14	US13	Overall predicted blanching (%)	Approximate standard error
	(no. of blanching replicates)				
PI 493547	4	3	3	51.3	3.27
PI 496448		3	3	49.7	4.45
PI 502111	4	3	3	49.0	3.12
PI 339960	4	3	3	47.7	3.12
PI 496401		3	3	46.8	4.43
PI 497517	4	3	3	46.0	3.11
PI 476025	4	3	3	45.2	3.11

565452, Hsi and Finkner 1972) and GenTex H & W 136 (released from the Borden Peanut Company, Portales, NM).

We report the results from 106 accessions of the USMC and the commercial varieties (US13 series). Commercial varieties were decided based on suggestions from USA peanut breeders, as well as inclusion of varieties grown on maximum area in the USA for each market type, including both high- and normal-oleic varieties. One accession (PI 288210) produced very little seed in all three replicates, so kernels were not available for blanching from those plots. The US13 trial therefore had 72 and 97 USMC accessions in common with AU13 and AU14, respectively (Table 2).

The three trials (AU13, AU14, US13) were harvested at various times based on different maturities of each of the genotypes present in the collection. Blanching assessments for all trials were carried out by using the newly developed protocol with 50-g samples and a 10-s abrasion time.

Entries from each of the AU13 and AU14 trials were blanched in Australia over 3 days, using three ovens each day. The ovens had eight racks with four shelves to accommodate the samples in a 2 by 2 arrangement. Entries from US13 were blanched in the USA over 5 days, using one oven at the Plant Genetic Resources Conservation Unit in Griffin, Georgia. The oven had five racks and each rack accommodated 35 samples in a 7 by 5 arrangement. All blanching designs were partially replicated designs (Cullis *et al*. 2006) to accommodate seed availability, and experiments were designed with the aim of achieving as much balance with the entries as possible to avoid confounding genotypic effects with positions in the ovens.

Phenotyping imported breeding lines from USA–APBP introduction series

The second series was a variety evaluation trial planted in 2015 at the QDAF Taabinga Research Station. This trial was conducted to assess blanching quality of elite US peanut lines imported under Materials Transfer Agreements (MTAs) from the peanut-breeding programs at the University of Florida at Marianna, Florida, North Carolina State University at Raleigh, North Carolina, and AgResearch Consultants, Ashburn, Georgia. The trial included two replicates of 36 entries: five local APBP checks, 29 lines imported from the USA, and two lines under development (not shown) from the APBP (Table 3). The trial was planted in two-row plots 5 m long with a 0.9-m inter-row spacing and seeding rate of ~12 plants m^{-1} following a Latinised row–column design. The trial was irrigated to prevent crop-water deficits, and crop management was according to best practice for soil nutrition, and pest and disease control. Blanching assessment was then carried out in field replicate order, using the APBP standard protocol of 200-g sample and 20-s abrasion time because seed availability was not limited.

Table 3. Predicted blanching percentages for the USA–APBP introduction trial, including 29 elite introductions from University of Florida ('UF' lines), North Carolina State University ('N' lines), AgResearch Consultants Inc. ('ACI' lines) and five Australian commercial check varieties (Kairi, Middleton, Holt, Fisher, Sutherland)
All genotypes had two replicates and standard error for all predictions was 2.95. Genotypes are sorted in order of descending blanchability

Genotype	Predicted blanching (%)
Kairi	92.9
N54	92.9
N51	92.9
UF70	92.3
ACI30	90.8
Middleton	90.7
N55	90.4
N50	90.3
Holt	90.2
UF69	90.0
N45	89.2
Fisher	89.1
UF66	88.9
ACI28	88.1
N47	87.9
N46	87.8
ACI27	87.8
ACI26	87.4
N53	87.3
ACI33	86.5
N48	86.5
ACI29	84.6
UF64	84.1
Sutherland	83.6
UF73	82.9
N44	82.4
ACI39	82.2
N57	81.6
UF72	81.1
N52	80.0
N49	78.6
UF65	77.4
ACI38	77.0
UF68	70.9

Table 4. Trials included in the APBP early-maturity series: description, design parameters, and trial dimensions
Trial code acronym comprises two digits for the year, the letter 'E' for early-maturity genotype series, and two letters for the location; 'G' indicates that the trial was at a grower's property, otherwise the location was a QDAF research station

Year	Location	Trial code	No. of genotypes	No. of replicates	No. of rows	No. of columns
2013	Bundaberg (grower property)	13E_BB_G	24	3	12	6
2013	North Queensland	13E_NQ	24	3	4	18
2013	Taabinga	13E_TB	24	3	8	9
2014	Bundaberg	14E_BB	24	3	8	9
2014	Bundaberg (grower property)	14E_BB_G	24	3	6	12
2014	Taabinga	14E_TB	24	3	6	12
2015	Bundaberg (grower property)	15E_BB_G	24	3	6	12
2015	Redvale	15E_RV	24	3	6	12
2015	Taabinga	15E_TB	24	3	6	12

Phenotyping APBP early-maturity lines

The third series included nine multi-location–year field experiments conducted during the 2013–15 to evaluate yield and quality performance of 45 early-maturity advanced breeding lines and three local checks (referred to as 'genotypes' for simplicity) as part of the regional trial series within the APBP (Tables 4 and 5). The different locations belong to Queensland's South Burnett region close to Kingaroy (QDAF Taabinga and Redvale Research Stations), the Coastal Burnett region (QDAF Bundaberg Research Station and a grower trial site) close to Bundaberg (24°52′S, 152°21′E), and the Atherton Tableland region (QDAF Kairi Research Station) in North Queensland near Kairi (17°1′S, 145°33′E). The same 24 genotypes were planted in all trials within the same year. Trials in 2013 had 16 and 9 genotypes in common with trials planted in 2014 and 2015, respectively. Trials planted in 2014 had 11 genotypes in common with those planted in 2015 (Table 5).

All trials were planted in two-row plots 5 m long with a 0.9-m inter-row spacing and seeding rate of ~12 plants m^{-1} in Latinised row–column designs with three replications. All trials were irrigated to prevent severe crop-water deficits, except for the 2015 Redvale trial (15E_RV), which received 450 mm in-crop rainfall and did not suffer significant crop-water deficits during crop growth (Table 4). Crop management of all trials was according to best practice for soil nutrition and pest and disease control.

Blanching assessment was carried out by using the APBP standard protocol of 200-g sample and 20-s abrasion time because seed availability was not a limiting factor. Blanching was conducted in field replicate order for all nine trials.

Statistical analyses for all trials

Statistical analyses of all individual field experiments followed the procedure outlined by Gilmour *et al*. (1997) to determine whether it was necessary to include terms to account for spatial variability. Additionally, trials in the USMC and APBP early-maturity series were combined into two analyses (one for each series) following the multi-environment trials method of analysis described by Smith *et al*. (2001). This method combines data for a set of environments (determined as combinations of location, year, and management—generally synonymous with experiments) where a common set of genotypes was grown with the main objective of providing reliable and accurate predictions of genotype performance across the environments as well as information on the interaction of genotypes with environments (G×E interactions).

The linear mixed model of Smith *et al*. (2001) includes environment as a fixed effect and genotype as a random effect, incorporates design effects and terms for spatial variability for each experiment, accommodates between-environment residual variance heterogeneity, and adopts a multiplicative factor analytic (FA) model for the G×E effects. The FA model allows for different genotypic variances between environments and different genotypic correlations across pairs of environments and does not require all genotypes to be tested in all environments.

Angular transformation, $\text{asin}\sqrt{(\text{blanching\%}/100)}$, was used in the analysis of the USMC and APBP early-maturity series to meet residual assumptions of the analyses (normality and homogeneity of residuals variances) within each trial. No transformation was required for the USA–APBP introduction trial.

All analyses were performed by using ASReml-R (Butler *et al*. 2009), and empirical best linear unbiased predictions (E-BLUPs) for blanching percentage across all environments, i.e. a predicted overall blanching percentage, and their standard errors were obtained for each genotype in each series. For series where angular transformation was used, back-transformed overall blanching percentage E-BLUPs and back-transformed standard errors (Jørgensen and Pedersen 1998) were presented; otherwise blanching percentage E-BLUPs are presented accompanied by their standard errors.

Broad-sense heritability (H^2) for each trial was calculated as follows (Cullis *et al*. 2006; Piepho and Möhring 2007):

$$H^2 = 1 - (\text{avsed}^2/2\sigma_g^2)$$

where avsed^2 is the square of the average standard error of difference and σ^2_g is the genotypic variance, both obtained from the model fitted to the data from each trial.

Results and discussion

Development of a phenotyping protocol for blanchability for limited seed availability

Early-generation selection is desirable and critical for maximising efficiency in breeding programs for any trait. Yet,

Table 5. Number of replicates for each genotype in each of the APBP early-maturity series trials in each year, with predicted overall blanching percentage in descending order and approximate standard errors, obtained from the factor analytic (FA2) model fitted to each trial

See Table 4 for trial codes. Local check varieties are Redvale, Tingoora and Walter; also noted is soon-to-be-released variety Taabinga

Genotype	13E_BB_G 13E_NQ 13E_TB	14E_BB_G 14E_BB 14E_TB	15E_BB_G 15E_RV 15E_TB	Predicted overall blanching (%)	Approx. standard error
P52-p199-80			3	94.6	0.93
P52-p206-90			3	94.1	0.97
Redvale	3	3	3	93.8	0.62
P51-p168-58			3	93.3	1.04
P52-p197-76			3	93.1	1.05
D291-p137-109		3	3	92.9	0.79
Tingoora	3	3	3	92.6	0.67
Walter	3	3	3	92.6	0.68
P13-p21-223	3			91.0	1.35
P23-p171-85	3	3		90.7	0.95
P52-p197-75			3	90.6	1.21
P52-p216-92			3	90.5	1.21
P13-p69-241	3			90.4	1.39
P52-p194-69			3	90.3	1.23
P20-25-p69-34			3	90.2	1.23
P23-p153-63 (Taabinga)	3	3	3	89.5	0.79
P19-4-p9-8		3		87.8	1.48
P19-1-p2-1	3	3		87.6	1.08
P49–15-p66-39			3	86.7	1.40
D291-p186-191		3		85.8	1.58
P13-p23-233	3	3	3	85.5	0.91
P23-p153-62	3			85.1	1.68
P19-4-p19–18		3		84.5	1.63
P20-25-p69–31			3	84.4	1.50
P52-p197-77			3	82.9	1.56
P23-p157-64	3			82.5	1.80
P23-p165-74		3		82.4	1.72
P24-p187/205-98	3	3	3	81.9	0.99
P23-p172-87	3			81.6	1.83
P24-p185-91	3	3	3	81.5	1.00
P20-25-p69-33		3	3	80.6	1.22
P54-52-p268-145			3	80.4	1.64
P23-p171-83	3	3		80.0	1.31
P23-p157-67	3			79.6	1.90
P23-p157-68	3	3	3	79.4	1.04
P22-63-p126-48		3		79.3	1.83
P19-1-p2-3	3	3	3	79.2	1.05
P13-p45-235	3	3		75.9	1.40
P19-4-p19-17	3	3		75.6	1.41
P52-p179-64			3	74.4	1.81
D288-1-p8-11	3			72.4	2.11
P23-p157-65		3		70.4	2.06
P24-p187/205-97	3	3		70.3	1.50
P13-p07-219	3	3		57.6	1.62
P24-p188-93	3			46.6	2.36

for determining blanchability a significant quantity of seed is required, which in practice has been very difficult to achieve in early generations given that most single plants produce only ~50–300 g seed, and most of this seed is needed to plant the line for the next generation. As such, phenotyping and selection for blanchability has typically only been conducted in later generations when seed quantities are more plentiful, which means a breeder may have to wait multiple generations to determine whether a new line meets the industry standard of >85% blanchability. Therefore, our objective was to develop a protocol in which blanchability could be accurately assessed, and which was comparable to commercial blanching standards on small quantities of seed and hence open up the possibility of early-generation selections. This was accomplished in two

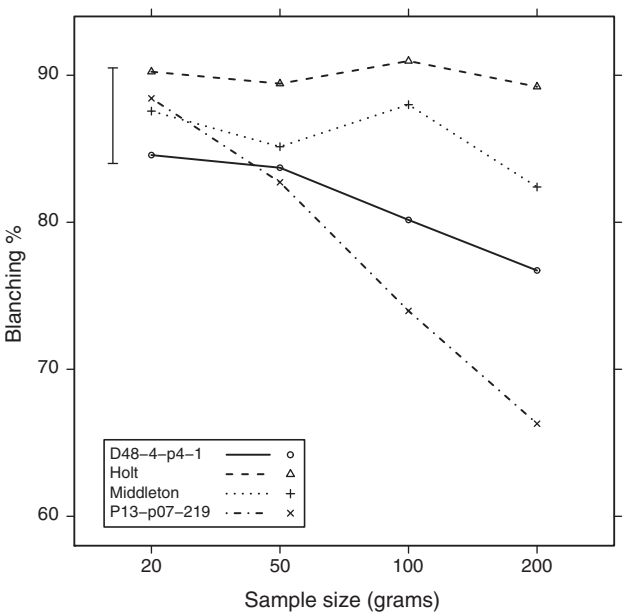

Fig. 1. Phenotyping protocol study, Expt 1: treatment means and l.s.d. ($P = 0.05$) for blanching percentage as a function of sample size and genotype. Blanching percentage was not significantly different among the four genotypes (l.s.d. = 6.5%) for 20-g samples; only the best (Holt) and the poorest (P13-p07-219) blanchers were significantly different for 50-g samples; average and poor blanchers had very low blanching percentage for 200-g samples.

experiments by using genotypes with contrasting blanching and subsequently assessing the effect of varying seed sample sizes and abrasion times in the blancher.

In Expt 1, blanching percentage was investigated as a function of genotype blanching quality (good, average and poor) and sample size (20–200 g) (Table 1), with all samples exposed to 20-s abrasion time. The genotype × sample size interaction was highly significant ($P = 0.002$), indicating that genotype blanchability was differentially affected by sample size (Fig. 1, l.s.d. = 6.5%). Genotypes were not significantly different from each other when 20-g samples were used (85% for D48-4-p4-1, ~88% for Middleton and P13-p07-219, and just over 90% for Holt). For the 50-g sample size, Holt (89%) and P13-p07-219 (83%) were the only genotypes significantly different from each other. For the 100-g samples, there was no significant difference between the two commercial varieties (Holt 91% and Middleton 88%) or between the breeding lines (D48-4-p4-1 80% and P13-p07-219 74%); thus, commercial varieties had significantly higher blanching percentage than breeding lines. For 200-g samples, Holt had a significantly higher (89%) and P13-p07-219 a significantly lower (66%) blanching percentage than the rest. The other two genotypes were not significantly different from each other (Middleton 82% and D48-4-p4-1 77%). Holt showed stable blanching percentage independent of sample size (range 89–91%). Both breeding lines, with average and poor blanching, showed significantly higher blanching percentage with smaller sample size (20 and 50 g) than with larger sample size (100 and 200 g). Lines P13-p07-219 had the most extreme response, ranging from 88% with 20-g samples to 66% for 200-g samples.

This strong genotype × sample size interaction may have been due to kernels in the smaller sample sizes receiving more effective abrasion time on the Ashton whole-nut abrasive roller. By contrast, kernels in the larger 200-g samples would have spent less abrasion time in contact with the roller because they would have been piled on top of each other for longer in the sample-holding chamber above the roller. Hence, kernels with poor blanching characteristics (breeding lines D48-4-p4-1 and P13-p07-219) with sample sizes of 20 and 50 g would likely have received more effective abrasion time resulting in higher blanching percentages. This sample-size effect would not have been as evident in the commercial varieties Holt and Middleton because testas would have been more easily removed anyway during the blanching procedure owing to their superior blanching characteristics.

The hypothesis of sample-size effect was tested in Expt 2 by considering the combined effects of genotype blanchability (good, poor), sample size (50 g, 200 g) and abrasion time (5–40 s) on blanching percentage (Table 1), with the aim of determining the optimum combination of factor levels required for development of an effective phenotyping protocol using smaller sample sizes. All 2-way interactions were significant (not the 3-way interaction); therefore, the means presented in Fig. 2 for a particular interaction are averaged over the levels of the third factor. The interactions of genotype × abrasion time and genotype × sample size were highly significant ($P < 0.001$), indicating that genotypes were, on average, differentially affected by sample size and abrasion time. The interaction sample size × abrasion time was also significant ($P = 0.043$), indicating that at least one combination of sample size by abrasion time produced significantly different blanching percentages from the others.

On average, Holt did not show significant differences in blanching percentage between the two sample-size levels (88% for 50 g and 85% for 200 g) across abrasion times, whereas P13-p07-218 showed a significantly higher blanching percentage for samples size 50 g (73%) than 200 g (59%) (Fig. 2*a*, l.s.d. = 3.4%). With respect to abrasion time (Fig. 2*b*, l.s.d. = 4.8%), Holt had a significantly lower blanching percentage, on average, at an abrasion time of 5 s (76%) than at other abrasion times (88% at 10 s, 91% at 20 s, and 92% at 40 s), which were not significantly different from each other. On the other hand, P13-p07-218 showed significantly increasing blanching percentages, on average, as abrasion time increased, except between 20 s (78%) and 40 s (81%). These results support the hypothesis that sample size and abrasion time can differentially affect the blanchability value of a genotype, especially poorer blanching genotypes, for which blanchability can increase significantly when using smaller sample sizes owing to a higher incidence of kernel abrasion around the roller.

Averaged across genotypes (Fig. 2*c*, l.s.d. = 4.8%), an abrasion time of 5 s resulted in significantly lower blanching percentage than the other abrasion times for both 50- and 200-g sample sizes, with 200-g samples showing significantly lower blanching percentage than 50-g samples. By contrast, abrasion times of 20 s and 40 s resulted in higher blanching percentages, which tended to be similar between sample sizes of 50 g and the industry standard 200 g, with only the 50-g

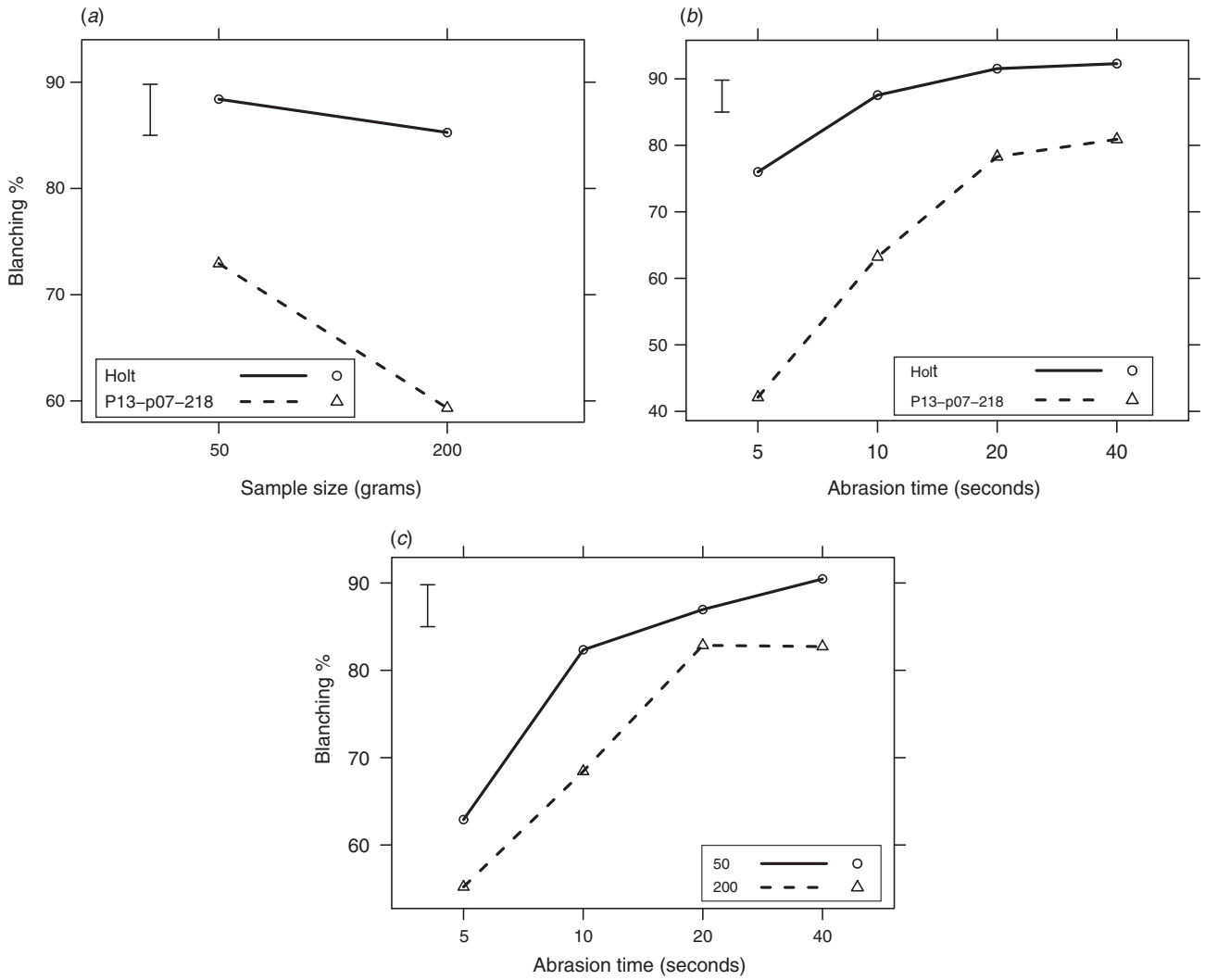

Fig. 2. Phenotyping protocol study, Expt 2: blanching percentage as a function of sample size, genotype and abrasion time. Treatment means and l.s.d. ($P = 0.05$) included in all two-way interactions: (*a*) genotype × sample size (l.s.d. = 3.4%), (*b*) genotype × abrasion time (l.s.d. = 4.8%), and (*c*) sample size × abrasion time (l.s.d. = 4.8%).

sample size at 40-s abrasion time significantly higher than the other combinations. The combination of 50-g sample size with 10-s abrasion time arguably produced the maximum discrimination for blanching percentage between genotypes and, importantly, was not significantly different from the standard protocol using 200-g samples with 20 s in the blancher.

These results therefore indicate that accurate phenotypic assessment for blanchability can be achieved by using smaller sample sizes than the current method (50 vs 200 g), provided abrasion time is reduced (10 s). This new protocol (50 g with 10 s abrasion time) opens up the possibility of obtaining blanching assessment in single plants and hence the option of screening for blanching in segregating populations in early generations where seed quantities are often limited. It is common to grow early-generation (F_2 and F_3) plants at wider spacing, which results in kernel yield in the range of 100–300 g per plant; thus, a small 50-g subsample for blanching assessment is now possible and it would allow single-plant selection and subsequent culling in a population for suboptimum blanching percentage. This newly validated blanching protocol was subsequently employed for assessing the genetic variability of various peanut germplasm sets grown in multiple environments, in order to evaluate G×E interactions and the broad-sense heritability of this important seed-quality trait.

Assessing genotypic variability and G×E interaction for blanchability in multiple germplasm sets

USMC series

The USMC peanut germplasm set represents ~1% of the entire genetic diversity in the USDA peanut germplasm collection and consists mostly of landraces (Holbrook and Dong 2005; Chen *et al*. 2014). Use of this USMC collection of 107 accessions grown in multiple environments provided a unique and representative diverse germplasm pool on which to assess accurately the extent of genetic diversity and estimate

Table 6. Summary of multi-environment trial analysis of the US mini core series

Trials did not have significantly different blanching percentage means ($P = 0.074$). All trials had high heritabilities. The US13 trial's genotypic variance was the least explained by the factor analytic (FA1) model; it also had a higher residual to genetic variance ratio and therefore a slightly lower heritability

Trial	Average blanching (%)	% Genotypic variance explained by FA1 model	Genotypic variance	Residual variance	Heritability
AU13	77.3	71	0.0120	0.0034	0.933
AU14	76.0	93	0.0255	0.0047	0.927
US13	69.6	65	0.0434	0.0166	0.886

the heritability for the blanching trait. Phenotypic measurements of blanchability were made across three multi-location–year trials (AU13, AU14, US13), which allowed an initial assessment of the extent of G×E interaction for this trait.

The analytical models used in the analysis of individual AU13 and AU14 trials included design terms for day, oven within day, and rack within oven within day (all as random effects), which when added up only represented 1% and 5% of the total variability in the respective trials. The model for the US13 trial included design terms for day, run within day, and rack within run as random effects, which when added up represented 17% of the total variability. The rack variance component was larger for the US13 trial than for the Australian trials (11% vs 0% and 1%); this was due to higher temperature variability within the oven used in the US experiment. Genotype effects represented 77%, 80% and 60% of the total variability for AU13, AU14 and US13, respectively, with residual variability representing 22%, 15%, and 23%, respectively.

The combined multi-environment analysis of this USMC series used an FA model with one factor (FA1), and explained 71%, 93% and 65% of the genotypic variability in blanching percentage for AU13, AU14 and US13, respectively (Table 6). The US13 trial had a higher proportion of residual to genotypic variance than the Australian trials, which was reflected in its slightly lower heritability (Table 6). The effect of the environment was not significant ($P = 0.074$) for this series, indicating that none of the trials had a mean blanching percentage significantly different from the rest. Generally, genotypic correlations were high and positive between pairs of trials: 0.81 between the Australian trials, and 0.68 and 0.77 between the US13 and the AU13 and AU14 trials, respectively. This indicated that for these trials, there was a high agreement in the ranking of the accessions and a low level of G×E interaction across years and locations, and that an overall prediction for blanching percentage across the three trials was an adequate measure for overall blanchability.

There was large genotypic variation in blanching percentage in the mini-core collection, which ranged from 45.2% to 94.4% (Table 2); thus, ~77% of the USMC accessions had an overall blanching percentage less than the industry blanching standard of 85% (Table 2). Because the USMC is genetically a diverse set selected from a genebank collection that contains many heirlooms, the range of variability among genotypes would be expected to be higher than in advanced breeding lines. Peanut breeders can therefore use this new mini-core information to select accessions as parents to develop new peanut cultivars with desirable blanching characters, or at least be aware if they are using a particular accession for another trait besides blanching that breeding populations may segregate widely for blanching characteristics.

Blanchability in USA commercial peanut varieties

As part of the US13 trial comprehensively cataloguing traits from the USMC collection, commercial varieties were included as checks for blanching. These commercial varieties allowed the variability of blanching to be assessed for lines currently grown for processing in the USA. Results for the 14 commercial varieties from the USA, representing the four major market types currently grown there, are presented in the top section of Table 2. These results were recorded from only one site (US13), although with multiple replications within the one site; however, they show that blanching varied widely among commercially grown varieties from 53.4% to 93.1%, with 36% of them not meeting the Australian industry standard of 85% blanchability. These data strongly suggest that blanching may not be a key quality trait targeted for selection in many US peanut-breeding programs.

Imported elite USA breeding lines–APBP introduction series

Multiple peanut germplasm sources were assessed for the blanching trait and estimated heritability, including a set of 29 recent elite/near-to-release imported US breeding lines, in a separate APBP trial in Australia. In theory, these elite lines should be less variable than the USMC collection. The analytical model used for this trial included replicate as a random effect, which was very small (variance component 0.81) and had a high ratio of genotypic to residual variance (36.4 vs 21.2). The trial did not require terms to adjust for spatial variability or autoregressive models for the error variances, indicating that spatial variability from the field could not be detected in blanching percentage. Unexpectedly, blanching percentage of the 29 introduced lines again varied widely from 70.9% to 92.9%, with 41% of these lines not meeting the 85% industry blanching standard (Table 3). Although the range of variation was higher than expected, it was lower overall than the USMC series.

Considering that peanut butter made with blanched kernels is one of the most common products from the US peanut crop, it is surprising that blanchability is not given more attention by peanut breeders and processors there. Interestingly, breeding lines tested from the three US breeding programs (University of Florida, North Carolina State University, and ACI Seeds) had both acceptable and unacceptable blanching types, indicating that each breeding program has developed lines of variable blanching quality. The breeders from these three peanut-breeding programs also confirmed that phenotyping for blanching and related selection were not routinely practiced in their programs, apart from limited blanching assessment on near-to-release lines entered in state or regional trials and before

decisions are made for commercial release. The main quality-related breeding objectives are high oleic fatty acid and flavour (Balota *et al*. 2016; B. Tillman, T. Isleib, K. Moore, pers. comm.). More recently, US peanut breeders have utilised wider sources of genetic resistance from the US peanut germplasm collection for important diseases such as *Tomato spotted wilt virus* (Culbreath *et al*. 2005; Tseng *et al*. 2016); hence, it is likely that there has been unintentional introgression of genes for poorer blanching characteristics into newer near-to-release cultivars. The high heritability recorded in the present study (Table 6), combined with the ability to conduct effective phenotyping for the blanching trait on small kernel samples as reported here, will therefore open up possibilities for future breeding and selection for this quality trait in US and other global peanut breeding programs.

It is relevant that the majority of peanut butter manufacturers in the USA blanch peanuts after roasting, rather than blanching pre-roast as is more widely practiced by Australian peanut butter manufacturers. Poorer blanching genotypes, as determined using the pre-roast blanching method, may have more acceptable blanching characteristics with the more aggressive post-roasting treatment. This could explain the lack of market signals back to US peanut breeders on the need to select for high blanching genotypes.

APBP early-maturity series

A detailed assessment of the G×E interaction for blanchability was made in a series of multi-year–site variety evaluation trials assessing blanching characteristics of elite early-maturing breeding lines produced by the APBP, including 45 advanced breeding lines and three local checks. These nine trials were conducted across highly contrasting geographic (North vs southern Queensland), cultural (research station vs grower farms) and temporal (2013–15) conditions. The analytical model for each individual trial in this series included replicate as a random effect (generally very small or zero), genotype effect and a residual term. None of the trials required terms to adjust for spatial variability or autoregressive models for the error variances, indicating that spatial variability from the field could not be detected in blanching percentage.

Analysis of the nine trials combined used an FA model with two factors (FA2). This model explained 62–100% of the genotypic variability in blanching percentage for the individual trials (Table 7), which was similar to the results observed in the mini-core trials. Genotypes had significantly different blanching characteristics, with predicted overall blanching percentage varying between 46.6% and 94.6% (Table 5). As expected, local check varieties all had high blanching percentage, with elite breeding lines varying widely. Large differences in blanching percentages were observed in very closely related genotypes. For example, the five P13 lines developed from the same parental cross had blanching percentages ranging from 57.6% to 91.0%.

Generally, genotypic correlations were high and positive between pairs of trials of this series, ranging between 0.55 and 0.99 (Fig. 3), indicating very low G×E across years and locations, a high agreement in the genotype rankings, and that an overall prediction for blanching percentage across trials was an adequate measure for overall blanchability for each genotype. The only exception was the 13E_NQ trial, where the model explained only 62% of the genotypic variability, and genotypic correlations were not as high as among the other trials (Fig. 3). This trial was affected by severe late leaf spot (caused by *Phaeoisariopsis personata*), a leaf disease that caused differential canopy senescence among genotypes depending on their relative susceptibility. Kernel development may have therefore been differentially affected among genotypes, thus causing environmentally induced, rather than genetic, differences in blanching. Similar environmental effects on genotypic ranking for blanching with severe end-of-season drought stress have been observed (G. C. Wright, unpubl. data); however, under most standard environmental conditions seen in this study, genotypic ranking for blanching appears to be highly conserved. Low G×E interaction for the blanching trait has been reported elsewhere (Mozingo 1979; Cruickshank *et al*. 2003).

Heritability and selection for blanchability

Evaluation of multiple germplasm sources in multiple environments demonstrated that the G×E effect on blanching was low. Heritability was subsequently estimated to evaluate how much of the trait can be explained by genetic variation. Very high heritability estimates for blanchability were observed in all 13 trials in this study and ranged from 0.742 to 0.967

Table 7. Summary of the multi-environment trial analysis of the APBP early-maturity series

See Table 4 for trial codes. The factor analytic (FA2) model captured a high proportion of the genotypic variability for most trials, except 13E_NQ, which had been affected by late leaf spot such that the genotype rankings followed a slightly different pattern from the other trials. All trials had high heritabilities

Trial	Average blanching (%)	% Genotypic variance explained by FA2 model	Genotypic variance	Residual variance	Heritability
13E_BB_G	86.7	100	0.0128	0.0026	0.941
13E_NQ	73.9	62	0.0137	0.0082	0.848
13E_TB	78.9	87	0.0315	0.0037	0.967
14E_BB	81.4	100	0.0134	0.0038	0.911
14E_BB_G	86.0	100	0.0108	0.0040	0.887
14E_TB	77.6	91	0.0192	0.0055	0.901
15E_BB_G	83.7	92	0.0200	0.0028	0.941
15E_RV	91.2	100	0.0060	0.0043	0.742
15E_TB	87.0	81	0.0166	0.0047	0.890

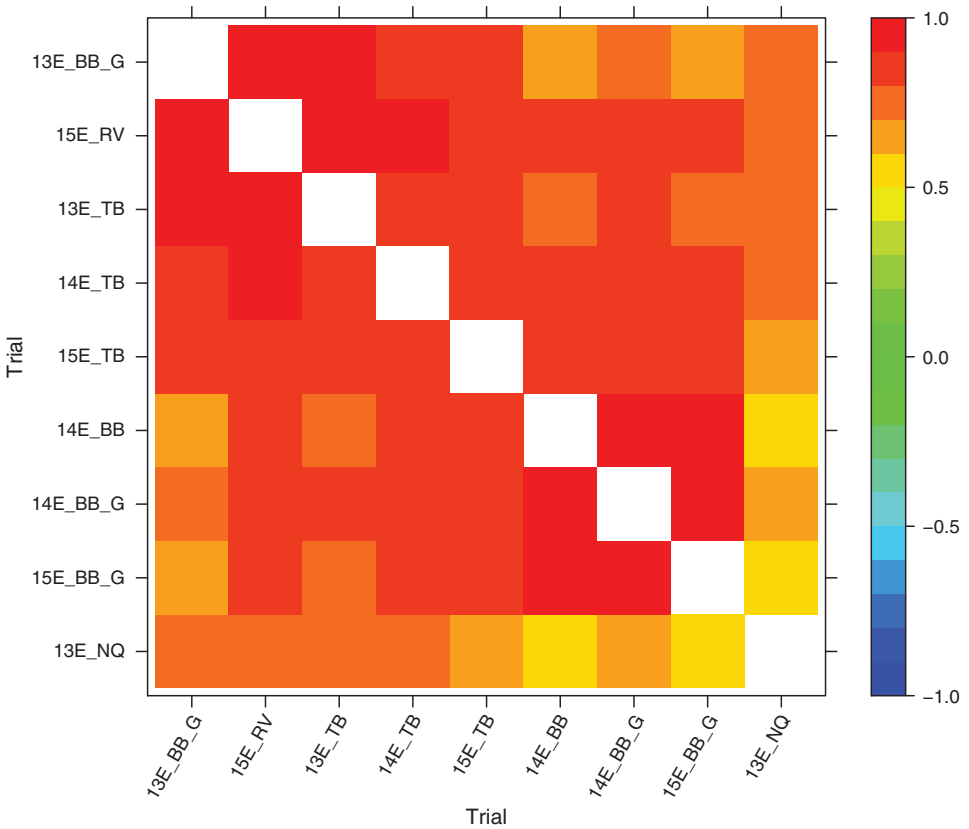

Fig. 3. Heatmap of the genotypic correlation matrix for blanching percentage in the APBP early-maturing series of trials. The scale on the side of the figure indicates the magnitude and direction of the genotypic correlations. Red–orange indicates strong positive correlation (i.e. strong agreement in genotype rankings) between pairs of trials. Dark shades of blue indicate strong negative correlation (i.e. strong disagreement in genotype rankings). Yellow, pale blue and green indicate very weak or no correlation between pairs of trials. The only trial with a slightly weaker genotypic correlation with the rest was 13U_NQ.

(Tables 6 and 7; heritability for the APBP introduction trial 0.774). These are the first reported heritability estimates for this trait in peanut. The consistently high heritability across environments combined with the large genotypic variability and low G×E interaction indicate that selection for blanchability should be highly effective in a limited number of environments, and be possible in early generations.

The trait is likely to be controlled by a major gene (or genes), and based on an early-generation selection experiment by Cruickshank *et al*. (2003), it was considered to be under oligogenic control. Shokraii *et al*. (1985) reported that poor blanchability is controlled by a dominant or semi-dominant gene; however, no data were presented in their paper to confirm this claim. Cruickshank *et al*. (2003) demonstrated very good response to selection from blanchability measured in kernels sampled (300 g) from bulk $F_{2:3}$ rows, but suggested that the significant quantity of seed required for the blanchability test precluded the option of single-plant selection in early generations or in a recurrent backcrossing program. Because understanding the underlying genetics of any trait is critical for improving the efficiency of the breeding process, segregating populations should be evaluated to determine how many genes are involved and confirm whether it is under oligogenic control.

This study, which assessed multiple germplasm sets in different environments, has demonstrated that accurate phenotyping for blanchability on single segregating plants is feasible with the use of a smaller 50-g kernel sample, and thus it presents the option of identifying good blanching phenotypes in pedigree or in single-seed-descent programs. In addition, the ability to phenotype single plants within recombinant inbred line populations opens up the possibility for accurate and rapid phenotyping of blanchability in genetic mapping studies aimed at developing new molecular markers for this trait, along with identifying the genes controlling it. Further, the high heritability and low G×E interaction effect demonstrate relative stability for this trait, which is important when moving commercial lines into new production areas.

Conflicts of Interest

The authors declare no conflicts of interest.

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