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Oil, Sugar, and Starch Characteristics in Peanut Breeding Lines Selected for Low and High Oil Content and Their Combining Ability

THOMAS G. ISLEIB,[†] HAROLD E. PATTEE,*[,‡] AND FRANCIS G. GIESBRECHT[§]

Department of Crop Science, North Carolina State University, Box 7629, Raleigh, North Carolina 27695-7629, Agricultural Research Servive, U.S. Department of Agriculture, Department of Botany, North Carolina State University, Box 7625, Raleigh, North Carolina 27695-7625, and Department of Statistics, North Carolina State University, Box 8203, Raleigh, North Carolina 27695-8203

Peanut seeds contain approximately 50% oil on a dry weight basis, making them a high fat food. Reduction of the oil content would make peanuts a more desirable food to fat conscious consumers. Removal of existing oil by processing is not feasible for in-shell peanuts, the dominant product of the North Carolina−Virginia area. To reduce oil content in in-shell peanuts, a genetic solution must be found. However, while reduced oil content is a desirable objective, changes in oil must not be accompanied by significant decreases in any of the desirable aspects of peanut flavor. Because the impact of selection for low or high oil on flavor is not known, it would be useful to know in what form dry matter is being stored in the seed, particularly if it is not being stored as oil. Screening of 584 accessions identified two lines (PI 269723 and PI 315608) with high and two (Robusto 2 and Robusto 3) with low oil contents, each pair differing in sugar content. The four parents were crossed in diallel fashion to investigate patterns of inheritance. General combining abilities (GCA) for oil content closely followed values of the parental lines. One low oil parent (Robusto 2) had a correspondingly elevated GCA for sugar content, but neither low oil parent had the effect of elevating starch in progeny. Reciprocal cross differences were found for starch and sugar contents, suggesting influences of cytoplasmic genes on those traits. These lines serve as resource material for researchers interested in the genetic and physiological aspects of the oil−sugar−starch relationship in peanuts.

KEYWORDS: General combining ability; GCA; specific combining ability; SCA; maternal effect; reciprocal effect

INTRODUCTION

The composition of peanut (*Arachis hypogaea* L.) seed has received much more attention from the U.S. peanut industry in the past 10−15 years. Consumers in the U.S. have become more health conscious and have requested low fat foods. The food industry has thus put an emphasis on the advertisement of products as low fat, reduced fat, or light. Peanut seeds contain approximately 50% oil on a dry weight basis, making them a high fat food. Reduction of the oil content would make peanuts a more desirable food to fat conscious consumers. In Title 21 of the Code of Federal Regulations (21CFR101.62), the U.S. Food and Drug Administration established standards whereby a product can be labeled reduced fat only if it has one-quarter less fat than the normal product. Peanuts would have to be consistently below 37.5% oil to qualify as having reduced fat. The peanut processing industry has responded to consumer aversion to peanuts by developing products with reduced fat such as peanut spreads and honey-roasted peanuts with some oil removed. Removal of existing oil by processing is not feasible for in-shell peanuts, the dominant product of the North Carolina−Virginia area. To reduce oil content in in-shell peanuts, a genetic solution must be found. Jakkula et al. (*1*, *2*) described a shriveled mutant peanut with low oil content. Breeding lines from this mutant vary in oil content averaging as low as 320 g kg^{-1}. In India and Korea, accessions with oil contents of 370 and 400 g kg^{-1} have been reported (*3*, *4*). A screening of selections from hirsuta landraces imported from Mexico found five selections with low oil content (*5*, *6*).

However, while reduced oil content is a desirable objective, changes in oil must not be accompanied by significant decreases in any of the desirable aspects of peanut flavor. Because the impact of selection for low or high oil on flavor is not known, it would be useful to know in what form dry matter is being stored in the seed, particularly if it is not being stored as oil. Plants could store carbon in any of several alternate forms, e.g.,

* To whom correspondence should be addressed. Tel: 919-515-6745. Fax: 919-515-7760. E-mail: harold_pattee@ncsu.edu.
[†] Department of Crop Science, North Carolina State University.
[‡] Agricultural Research Servive, U.S. Department of Agriculture.
[§] Department of Statistics, North Carolina State University.

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Table 1. Low and High Oil Lines Identified in a Screen of 584 Accessions Composing the NCSU Peanut Germplasm Collection

GP no.	NC accession no.	identity	oil content (g kg^{-1} dry weight) 1990	1995	adjusted mean	standardized mean
		low oil lines				
2866	02343	Robusto 1 (selection from cross between two irradiated NC 4 leaflet mutants)	461	422	442	–3.51
2732[a]	02237	Robusto 2 (selection from cross between two irradiated NC 4 leaflet mutants)	444	450	447	–3.21
2493[a]	02237	Robusto 3 (selection from cross between two irradiated NC 4 leaflet mutants)	441	457	449	–3.11
3075	02347	Robusto 4 (selection from cross between two irradiated NC 4 leaflet mutants)	470	435	452	–2.92
2154	02254	Robusto 5 (selection from cross between two irradiated NC 4 leaflet mutants)	454	453	454	–2.85
2726	02240	Robusto 6 (selection from cross between two irradiated NC 4 leaflet mutants)	466	443	455	–2.80
2151	02244	Robusto 7 (selection from cross between two irradiated NC 4 leaflet mutants)	472	454	463	–2.34
2805	02314	Robusto 8 (selection from cross between two irradiated NC 4 leaflet mutants)	482	445	464	–2.30
3072	01459	Corduroy Robusto (selection from cross between two irradiated NC 4 leaflet mutants)	480	448	464	–2.28
2755	02339	Robusto 9 (selection from cross between two irradiated NC 4 leaflet mutants)	499	431	465	–2.21
2744	02326	Robusto 10 (selection from cross between two irradiated NC 4 leaflet mutants)	467	465	466	–2.16
2753	02353	Robusto 11 (selection from cross between two irradiated NC 4 leaflet mutants)	480	455	468	–2.06
2776	02317	Robusto 12 (selection from cross between two irradiated NC 4 leaflet mutants)	471	466	469	–2.01
3910		CBR-R2	481	- -	469	–1.97
2555	02355	Robusto (selection from cross between two irradiated NC 4 leaflet mutants)	460	479	469	–1.96
		high oil lines				
2226	17065	A48/A48	555	524	540	+1.97
1093	03033	NC 3033	548	532	540	+2.00
3729	17133RF	Gregory Coll. no. 190	563	518	540	+2.02
1118	17941	NC 8C	553	530	541	+2.05
3963		Southern Runner	563	521	542	+2.08
3965		Sunbelt Runner	562	522	542	+2.12
3925		Georgia Red	563	525	544	+2.21
2122	02128	X12 B and BR Vigorous	559	530	545	+2.25
3249	10272	4508/Recurved F3	559	533	546	+2.33
2121	02125	BR-1 Vigorous	572	533	552	+2.67
3946	10191	PI 121067 (“Indio” from Misiones, Argentina)	559	549	554	+2.76
3698[a]		PI 315608 (“selection from U.S. introductions” from A. Ashri, Rehovoth, Israel)	579	533	556	+2.88
3942		PI 261893 (“Overo” from Cochabamba, Bolivia)	571	547	559	+3.05
0607	02551	Virginia Red	567	553	560	+3.10
0631[a]	02630	PI 269723 (“Virginia Red” from Israel)	583	540	562	+3.21

[a] Line selected for use as a parent in diallel mating.

sugars, complex carbohydrates such as starch, or proteins, although additional proteins would require additional nitrogen. Sugars made by photosynthesis in the leaves move into the seeds where they are converted to different energy-containing constituents. During early development, the peanut seed converts the incoming sugars primarily to starch, and during later maturation, the sugars are converted primarily to oil (*7*). Although no correlation between oil content and flavor has been reported in the literature, one might expect that altering oil content could have an impact on peanut flavor. Lines that store carbon as sugars would be expected to exhibit heightened sweetness, a desirable change in flavor, while those that stored carbon as starch might exhibit less sweetness. Sweetness is a highly heritable aspect of peanut flavor, and it is positively correlated with overall roasted peanut quality (*8*). The objectives of this study were (i) to find peanuts that accumulate sugars or starch rather than converting the sugars to oil and (ii) to obtain information on the genetic transfer of seed composition traits.

MATERIALS AND METHODS

Genotype Resources. A germplasm collection of 584 accessions maintained by the NCSU peanut breeding program was grown in unreplicated nurseries at the Peanut Belt Research Station at Lewiston, NC, in 1990 and 1995. This collection includes 100 released cultivars and germplasm lines, 17 U.S. landrace cultivars, 121 plant introductions, 55 breeding lines, and 297 selections from a program of mutation breeding conducted by W. C. Gregory et al. (*9*). All four of the U.S. market types (runner, Virginia, Spanish, and Valencia) and both subspecies and four out of six botanical varieties (*A. hypogaea* subsp. *hypogaea* var. *hypogaea*; subsp. *fastigiata* Waldron vars. *fastigiata*, *peruviana* Krapov. & W. C. Gregory, and *vulgaris* Harz) were represented. The 121 introductions included lines from South America, Africa, and South and Southeast Asia. Standard cultural practices were

Table 2. Mean Squares from ANOVA of Diallel Mating among Four Peanut Lines with Differing Composition

source	df	oil (g kg^{-1} dry weight)	starch (mg g^{-1})	sugar (mg g^{-1})
entries	13	10272.22^{c}	352.29^{b}	849.76^{c}
parents vs crosses	1	5774.22^{b}	76.80	312.39
among parents	3	6542.96^{c}	286.18	331.86
among crosses	9	11744.47^{c}	412.78^{c}	1094.42^{c}
GCA	3	22263.26^{c}	404.18^{b}	427.03
SCA	2	4410.87^{c}	241.70	51.94
reciprocal effects	4	2161.95^{b}	505.16^{b}	855.67^{b}
maternal effects	3	2505.50^{b}	580.11^{c}	478.87
specific reciprocal	1	1346.49	402.34^{a}	1471.33^{b}
error	30	789.13^{c}	128.13^{c}	296.08^{c}
duplicates	42	61.55	2.58	6.39

$^{a-c}$ Denote mean squares significance at the 10, 5, and 1% levels of probability, respectively, by *F* test.

employed in growing the collection, including fertilization and control of weeds, diseases, and insects. After mechanical digging of the plots at approximately 155 days after planting, pods were picked from the plants by hand and dried over forced air to approximately 9% moisture prior to shelling and storage at −20 °C.

Oil Analysis. For each grow out year, approximately 10 g of sound mature kernels (seeds that ride a 5.95 mm × 25.40 mm screen) was sampled from each of the 584 accessions and subjected to analysis of oil content by nuclear magnetic resonance (NMR). Samples were dried to near 0% moisture by placing them in a drying oven at 70 °C for 24 h. In the interval between drying and NMR assay, samples were placed in desiccators to prevent absorption of atmospheric moisture. NMR data were subjected to two way analysis of variance (ANOVA), and means were calculated across years.

Parent Selection. Following standardization of the means adjusted to a common year effect, lines deviating from the mean by 1.96 standard deviations or more were classified as having high and low oil content. Seeds from the 1995 grow out of 42 high and low oil lines were assayed for starch and sugar contents using the method of Pattee et al. (*7*). Four of the 42 lines were selected to be used as parents in a diallel mating. They were PI 269723 (high oil, low sugar), Robusto 2 (low oil, high sugar), Robusto 3 (low oil, low sugar), and PI 315608 (high oil, high sugar). The parents were crossed in all combinations in 1998. The four parents and all F_1 hybrids were grown in the greenhouse in the summer of 1999. Seeds were harvested from individual plants. To preserve viable embryos for future use, the distal half of each cotyledon was excised for analysis of composition.

Seed Composition. A 35 g sample of seeds harvested from each individual F_1 or selfed plant was ground in a Krupp grinding mill, and two duplicate 1 g samples were taken for analysis. Oil was extracted as described by Oupadissakoon et al. (*10*); the supernatant was decanted into aluminum pans and evaporated, and the oil residue was measured gravimetrically. Following removal of the oil, the sugar and starch were extracted from the residue and measured using the protocol of Pattee et al. (*7*).

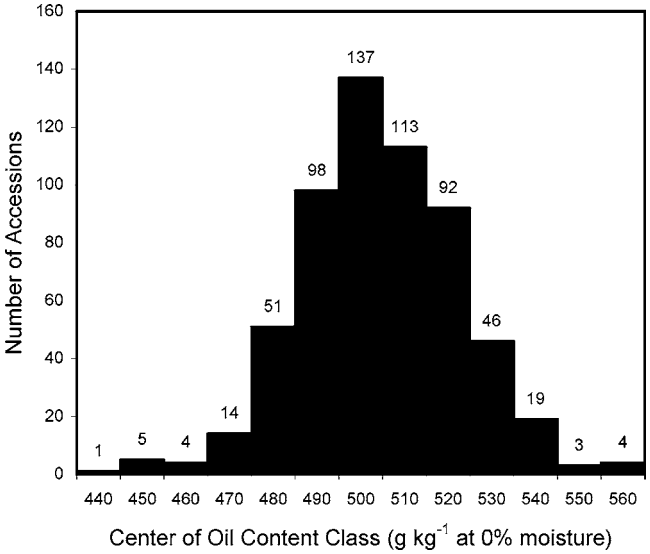

Figure 1. Distribution of oil content on a dry weight basis among 584 accessions of the NCSU peanut germplasm collection measured by NMR.

Statistical Analysis. Composition data were analyzed as a completely random design with unequal replication of treatments. Plant-to-plant variation was considered to be the experimental error with variation among duplicates an estimate of sampling error. Diallel analysis of the composition was performed using the general linear models procedure (PROC GLM) of SAS (*11*). A design matrix including all model effects was developed using the statistical model of Griffing (*12*) method 4 model I with partitioning of reciprocal cross-effects described by Cockerham and Weir (*13*). Effects of the parents as inbred lines per se were estimated separately from their effects in hybrid combination.

RESULTS AND DISCUSSION

The range of oil content in 584 lines in the NCSU peanut breeding program's germplasm collection was 442−562 g kg^{-1} on a dry weight basis (DW) with a mean of 504 g kg^{-1} and a standard deviation of 18 g kg^{-1}. The distribution was unimodal and asymmetrical (**Figure 1**). The lines with the lowest oil contents were primarily Robusto mutants selected from populations created by crossing different leaflet mutants generated by irradiating seeds of NC 4 (*14*, *15*) (**Table 1**). Several of the

Table 3. Line Effects, General Combining Abilities, Maternal Effects, and Predicted Effects of Lines Used as Female and Male Parents in Crosses

		mg g^{-1}				
trait	parent	line effect ($p_i \pm$ SE)	GCA effect ($g_i \pm$ SE)	maternal effect ($m_i \pm$ SE)	female effect ($g_i + m_i \pm$ SE)	male effect ($g_i - m_i \pm$ SE)
oil	PI 269723	58.0 ± 17.9^{b} a	29.8 ± 7.7^{b} a	−12.3 ± 4.4^{b} b	17.5 ± 8.9^{a} a	42.1 ± 8.8^{b} a
	PI 315608	40.1 ± 17.9^{a} a	22.2 ± 7.6^{b} a	−5.5 ± 4.7 ab	16.7 ± 9.2^{a} a	27.7 ± 8.7^{b} a
	Robusto 3	−52.4 ± 17.9^{b} b	−31.1 ± 8.6^{b} b	−2.2 ± 3.8 ab	−33.3 ± 10.1^{b} b	−28.9 ± 8.7^{b} b
	Robusto 2	−45.7 ± 17.9^{a} b	−20.9 ± 10.7^{a} b	20.0 ± 9.0^{a} a	−1.0 ± 18.4 ab	−40.9 ± 7.4^{b} b
starch	PI 269723	−5.4 ± 7.1 b	−1.6 ± 3.0 ab	4.6 ± 1.7^{a} a	2.9 ± 3.5 ab	−6.2 ± 3.5^{a}
	PI 315608	−5.6 ± 7.1 b	7.7 ± 3.0^{a} a	5.3 ± 1.8^{b} a	13.0 ± 3.6^{b} a	2.5 ± 3.4
	Robusto 3	17.9 ± 7.1^{a} a	−0.1 ± 3.4 ab	−1.4 ± 1.5 b	−1.5 ± 4.0 b	1.3 ± 3.4
	Robusto 2	−6.9 ± 7.1 b	−6.0 ± 4.2 b	−8.5 ± 3.5^{a} b	−14.4 ± 7.2^{a} b	2.5 ± 2.9
sugar	PI 269723	−10.8 ± 10.8	−5.4 ± 4.6	−0.2 ± 2.7	−5.7 ± 5.4	−5.2 ± 5.3 b
	PI 315608	−8.4 ± 10.8	−1.3 ± 4.6	5.0 ± 2.8	3.7 ± 5.5	−6.3 ± 5.3 b
	Robusto 3	1.7 ± 10.8	−4.0 ± 5.2	2.0 ± 2.3	−2.0 ± 6.1	−5.9 ± 5.3 b
	Robusto 2	17.5 ± 10.8	10.7 ± 6.5	−6.8 ± 5.4	3.9 ± 11.1	17.5 ± 4.5^{b} a

^{a,b} Denote effects significantly different from zero by *t*-test at $P < 0.05$ and $P < 0.01$, respectively. Effects within a column followed by the same letter are not significantly different by *t*-test ($P < 0.05$).

low oil lines were closely related. The high oil lines were more diverse in their origins, including mutants, improved cultivars, and several plant introductions.

Of the four lines selected as parents for the diallel mating, two (Robusto 2 and Robusto 3) had low oil content (447 and 449 g kg^{-1} DW, respectively), and two (PI 315608 and PI 269723) had high oil content (556 and 562 g kg^{-1} DW, respectively). Within each of these pairs, one member had slightly higher and one slightly lower sugar content (48.6 vs 43.6 mg g^{-1} for Robusto 2 vs Robusto 3 and 44.5 vs 40.5 mg g^{-1} for PI 315608 vs PI 269723). There was a large difference in starch content between Robusto 2 and Robusto 3 (42.8 vs 58.6 mg g^{-1}) but not between PI 315608 and PI 269723 (39.9 vs 36.5 mg g^{-1}).

Mean oil content of the four parents grown in the greenhouse as part of the diallel experiment was lower than observed in the field (426 vs 503 g kg^{-1}), but the correlation of greenhouse and field values across the four parents was nearly perfect ($r = 0.994$, $P < 0.01$). Variation in oil among the four parents grown in the greenhouse was significant ($P < 0.01$), but variation in sugar and starch content was not (**Table 2**) despite having chosen the parents for their apparent differences in sugar content. Nevertheless, there was significant variation among the crosses for all three composition traits (**Table 2**). Oil content exhibited significant effects of general combining ability (GCA) usually interpreted as reflecting large additive genetic effects, specific combining ability (SCA) usually associated with dominance effects, and maternal effects that are generally interpreted as being associated with cytoplasmic differences among parents. Starch content was affected by GCA and maternal effects. Sugar content was affected only by specific reciprocal effects reflecting interactions between nuclear and cytoplasmic genes. The presence of maternal and specific reciprocal effects for seed composition traits is somewhat unusual. Reciprocal differences have been reported for oil and starch content in maize (*16*), but the reciprocal effect for starch could be caused by differential contribution of maternal and paternal nuclear genes to the triploid endosperm that composes most of a maize seed. Most oil in maize seed is found in the germ, a diploid tissue that has equal contribution of nuclear genes from seed and pollen parents but that derives its cytoplasm from the seed parent. The bulk of peanut seed is diploid cotyledonary tissue. In peanut, reciprocal cross effects on oil content have been reported in crosses involving mutant lines with shriveled seed and greatly reduced oil content (*2*). Such differences can be due to direct effects of cytoplasm, suggested by maternal effects in the diallel analysis, or to interaction between cytoplasmic and nuclear genes, suggested by specific reciprocal diallel effects. Reciprocal cross differences have been reported previously in peanut for composition traits (*1*, *17*).

The presence of maternal effects for oil content suggests that one should pay close attention to the direction of the cross in using these four parents in crosses to modify oil content. The magnitudes of the effects exerted by high oil parents, PI 269723 and PI 315608, and low oil parent Robusto 2 were greater when used as males rather than as females (**Table 3**). The GCA effects of the four parents on oil content were very similar to their effects as lines per se. For any of the three composition traits, there was no difference in the GCA effects of high oil parents PI 269723 and PI 315608 or in their effects when used as either a female or a male parent in crosses. Robusto 2 caused a significant increase in sugar content when used as a male, and the increase was different from the effect of any other parent used as a male. Robusto 3 as a line per se had elevated starch content, but that elevation was not passed to progeny regardless of the direction of the cross. These results provide resource material for researchers interested in the genetic and physiological aspects of the oil−sugar−starch relationship in peanuts.

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