horticulturae

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Effect of Genotype-by-Environment Interaction on Oil and Oleic Fatty Acid Contents of Cultivated Peanuts

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Abstract: Twenty-seven genotypes of varieties and advanced breeding lines were grown in two locations in three years with three replications to estimate the effects of the genotype-by-environment interaction (G × E) on the oil and oleic fatty acid contents of cultivated peanuts. Oil and oleic fatty acid contents were quantified using NMR and GC, respectively. The tested lines were genotyped with functional SNP markers from the *FAD2A* and *FAD2B* genes using real-time PCR and classified into four genotypes. The results indicated that Alabama was the environment that better discriminated the test genotypes during the year 2012. Eight promising selected genotypes #12, #15, ARSOKR, Brantley, GaHO, M04-149, M04-48, and SunO97R showed wide adaptation and high-oleic acids of 83.02%, 81.32%, 82.03%, 81.15%, 79.21%, 80.94%, 82.46%, and 82.18%, respectively. The Additive Main Effects and Multiplicative Interaction (AMMI) model that combines the conventional analyses of variance for additive main effects with the principal component analysis (PCA) for the non-additive residuals was applied to estimate the additive effects from *FAD2A* and *FAD2B* genes and the G × E interaction. The results indicated significant G × E interactions for oleic fatty acid contents. No correlation between oil content and *FAD2A* and *FAD2B* genes was found. The *FAD2B* gene had a larger additive effect than the *FAD2A* gene. The results from this study may be useful not only for peanut breeders, but also for food processors and product consumers to select suitable cultivars.

Keywords: peanut; G by E; oil; oleic fatty acid

Citation: Zhang, H.; Yu, Y.; Wang, M.; Dang, P.; Chen, C. Effect of Genotype-by-Environment Interaction on Oil and Oleic Fatty Acid Contents of Cultivated Peanuts. *Horticulturae* **2023**, *9*, 1272. https://doi.org/10.3390/horticulturae9121272

Academic Editor: Luigi De Bellis

Received: 5 October 2023
Revised: 22 November 2023
Accepted: 23 November 2023
Published: 27 November 2023

1. Introduction

Peanut (*Arachis hypogaea* L.), an autogamous allotetraploid legume (2n = 4x = 40), is an important oilseed crop providing rich nutrients all over the world [1]. It has A and B subgenomes that came from their diploid ancestors *Arachis duranensis* (A-genome) and *Arachis ipaensis* (B-genome), respectively. Its seeds generally contain 50% oil, 25% protein, and some useful phytochemicals such as flavonoids, resveratrol, folic acids, and tocopherols [2–4]. The peanut oil quality was mainly affected by its fatty acid composition. Peanut oil has eight major fatty acids including oleic acid (C18:1), linoleic acid (C18:2), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), gadoleic acid (C20:1), behenic acid (C22:0), and lignoceric acid (C24:0), among which the amounts of only three fatty acids (C18:1, C18:2, C16:0) exceed 50% [5,6]. These three fatty acids constitute about 90% of peanut oil fat, while only C18:1 and C18:2 account for about 80% of peanut fatty acids. High O/L ratio (ratio of oleic and linoleic acid) is an important parameter for desired oil quality. Peanut with a high oleic acid content can offer a longer shelf life, flavor, and several health benefits such as a lowered risk of heart diseases and slowed atherosclerosis and tumorigenesis development [7,8].

Most of studies were conducted on breed peanut varieties with a high oleic acid content [9–11]. For example, one high oleate cultivar, SunOleic 95R, includes 49% oil composed of 80.6% oleic acid and only 2.8% linoleic acid [11]. In peanut, genes *ahFAD2A* and *ahFAD2B*, encoding enzyme fatty acid desaturase (FAD2), controls the quantity of oleic acid. The function of this enzyme is to convert oleic acid to linoleic acid in the fatty acid synthesis pathway, while the high-oleic mutants will appear if this enzyme is inactivated [12,13]. In 1987, Norden et al. first identified high-oleic mutants F435-2-1 and F435-2-2, and the content of oleic acid was up to 80%, while the linoleic acid level was only 2% [14]. The mutant *ahFAD2A* gene, with a G448A substitution, was located on the peanut A genome, and the *ahFAD2B* gene, with an insertion of one base pair in 442, was located on the peanut B genome [15–17]. Subsequently, F435 has been used to develop a series of breeding lines with a high-oleic-acid characteristic [11]. Other high-oleate mutants were produced through mutagenesis [18,19]. In addition, new mutation sites were also identified in *ahFAD2B* with a substitution of C301G from *Arachis hypogaea* [17] and in FAD2H with a substitution of C37T from *Arachis veigae* [20].

Oil and oleic acid contents in peanut seeds are complex quantitative traits that are controlled by more than one gene and are affected by the environment. Genotype, environment, and G × E interaction can affect the performance of the varieties. The environment allows the varieties to exhibit their best advantage. G × E analysis is the final step for the breeders to select the promising breeding materials [21]. Among the statistical techniques used to analyze and interpret G × E data, the additive main effect and multiplicative interaction (AMMI) and genotype-by-environment interaction (GGE) are two most popular analysis methods [22].

Until now, no research on the genotype-by-environment interaction on oil and oleic acid contents in cultivated peanut has been conducted. And before this study, we did not know which gene had a higher additive effect on oleic acid, considering the two genes *ahFAD2A* and *ahFAD2B*. The objectives of this research were to (1) determine the variability in oil and oleic acid among germplasm accessions; (2) determine the effects of the genotype x environment interaction on the oil and oleic acid contents; and (3) determine the additive effects of genes *ahFAD2A* and *ahFAD2B*.

2. Materials and Methods

2.1. Germplasm Selection and Fresh Seed Collection

A total of 27 accessions (#10, #12, #14, #15, #16, #16-1, ARSOKR, AT3085RO, Brantley, Exp27-1516, F435, F435HO, F435N, FR458, Fla-07, Florunner, Ga02C, GaGreen, GaHO, M04-0149, M04-048, M04-088, NC-7, Olin, SunOleic93R, SunOleic97R, WT4-121; Table 1), representing a wide range of oleic acid content variabilities (from low to high oleic acid content) were selected from the USDA cultivated peanut germplasm collection. In order to determine the effects of genotype (G, i.e., germplasm accessions), environment (E, i.e., locations and years), and G × E interaction, 240 seeds for each accession were requested from the USDA-ARS, Plant Genetic Resources Conservation Unit (PGRCU) in Griffin, GA. A total of 80 seeds of each accession were cultivated for three years at two locations (2010, Georgia; 2011, Alabama; 2012 Alabama). Twenty seeds were planted in two 10-feet-long rows for each replicate. Each accession had three replicates for each location. The planting and harvesting dates were determined according to the local varieties. After harvesting, the pods were dried and shelled by hand. Freshly harvested seeds (around 10% water content) from the fields were collected and used for chemical nutritional quality analysis.

Table 1. The genotypes of 27 accessions used in this study.

Variety	FAD2A	FAD2B	Genotype
#10	aa	bb	aabb
#12	aa	bb	aabb
#14	aa	bb	aabb
#15	aa	bb	aabb
#16	aa	bb	aabb
#16-1	AA	BB	AABB
ARSOKR	aa	bb	aabb
AT3085RO	aa	bb	aabb
Brantley	aa	bb	aabb
Exp27-1516	aa	BB	aaBB
F435	AA	BB	AABB
F435HO	aa	bb	aabb
F435N	aa	BB	aaBB
Fla-07	aa	bb	aabb
Florunner	aa	BB	aaBB
FR458	aa	bb	aabb
Ga02C	aa	bb	aabb
GaGreen	aa	BB	aaBB
GaHO	aa	bb	aabb
M04-149	aa	bb	aabb
M04-48	aa	bb	aabb
M04-88	aa	bb	aabb
NC-7	aa	BB	aaBB
Olin	aa	bb	aabb
SunO93R	aa	bb	aabb
SunO97R	aa	bb	aabb
WT4-121	aa	bb	aabb

The letters mean different genotypes.

2.2. Genotyping FAD2A and FAD2B through Real-Time PCR

Genotyping was carried out using functional SNP markers from the *FAD2A* and *FAD2B* genes through real-time PCR according to the published method. Peanut seed slices (75–150 mg) were put into a 2 mL microcentrifuge tube, along with 600 µL of P1 buffer from the Omega-BioTek kit (Doraville, GA, USA) and two 3 mm tungsten carbide beads (Qiagen, Valencia, CA, USA) for the purpose of extracting DNA. After that, the tissue was ground up for three minutes at 30 Hz using a Retsch Mixer Mill 301 (Leeds, UK). Using a DyNA Quant 200 fluorometer from Hoefer Pharmacia Biotech (San Francisco, CA, USA), extracts were measured. To assess the amount and caliber of each extraction, all samples were also placed onto a 1% agarose gel using a Low DNA Mass Ladder from Invitrogen (Carlsbad, CA, USA). The samples were then diluted to 10 ng/µL in order to perform real-time PCR. 1× TaqMan Genotyping Master Mix (Applied Biosystems), 0.16 µM forward primer, 0.16 µM reverse primer, 0.4 µM VIC probe, 0.3 µM 6FAM probe, and 0.4 ng/µL of DNA made up the 25 µL total volume of the PCR reaction. AmpliTaq Gold polymerase and ROX, a passive internal reference to account for signal variance between wells, are included in the TaqMan Genotyping Master Mix. Applied Biosystems' ABI StepOne real-time PCR equipment was used to conduct each PCR experiment. The cycling conditions were as follows: one cycle of 60 °C for 30 s, one cycle of 95 °C for 10 min, 50 cycles of 95 °C for 15 s and 62 °C for 1 min, and one final cycle of 60 °C for 30 s.

2.3. Oil and Fatty Acid Composition Measurement

Five medium-sized and healthy, naturally dried peanut seeds were crushed into fine powder in a small plastic bag with a hammer. Approximately 200 mg crushed seed powder was transferred into a small column and then pressed into a small pellet. Oil was quantified through nuclear magnetic resonance (NMR) analysis on a minispec seed analyzer (Bruker Optics Inc., Houston, TX, USA) according to the published report [23]. Fatty acid

methyl esters (FAMEs) were prepared from seeds through alkaline transmethylation, and fatty acid composition was determined using an Agilent 7890A gas chromatography (GC) equipped with a flame ionization detector (FID) and an autosampler. Sample preparation, GC operation, and data collection followed the standard methods routinely used by Wang's lab [23]. Genotyping was carried out using functional SNP markers from the FAD2A and FAD2B genes through real-time PCR.

2.4. Statistical Analysis

A mixed-model analysis of variance (ANOVA) was performed using the GLM procedure of SAS (SAS, 2008, Online Doc® 9.2., SAS Institute Inc., Cary, NC, USA) and means were separated using Duncan's multiple test procedure. Excel (2016) was used to perform variance analysis of variable components among 27 tested varieties. R4.0.3 [24] was used for AMMI and GGE analysis. Narrow-sense heritability (h^2) estimate for *FAD2A* gene can be estimated as $h^2_a = \sigma^2_a/[(\sigma^2_e/yab) + (\sigma^2_{yab}/yb) + (\sigma^2_{ya}/b) + (\sigma^2_{ab}/y) + \sigma^2_a]$, where σ^2_a is variance for gene *FAD2A*, σ^2_e is error variance, σ^2_{yab} is variance for *FAD2A* × *FAD2B* × year, σ^2_{ya} is variance for *FAD2A* × year, σ^2_{ab} is variance for *FAD2A* × *FAD2B*, y is number of years, and a is degrees of freedom. Narrow-sense heritability (h^2) estimate for *FAD2B* gene can be estimated as $h^2_b = \sigma^2_b/[(\sigma^2_e/yab) + (\sigma^2_{yab}/yb) + (\sigma^2_{yb}/b) + (\sigma^2_{ab}/y) + \sigma^2_b]$, where σ^2_b is variance for gene *FAD2B*, σ^2_e is error variance, σ^2_{yab} is variance for *FAD2A* × *FAD2B* × year, σ^2_{yb} is variance for *FAD2B* × year, σ^2_{ab} is variance for *FAD2A* × *FAD2B*, y is number of years, and b is degrees of freedom. Two-factor analysis of variance for estimates of two-gene model genetic effects was suggested by Cockerham [25] (Table 2).

Table 2. Two-factor analysis of variance for estimates of two-gene model genetic effects suggested by Cockerham (1963).

Source	Degrees of Freedom	Expected Mean Squares
Year (Y)	$(Y - 1) = 2$	
Rep (year)	$(R - 1) \times Y = 6$	
Fad2A (A)	$(A - 1) = 1$	$\sigma^2_e + 3\sigma^2_{yab} + 6\sigma^2_{ya} + 9\sigma^2_{ab} + 18\sigma^2_a$
Fad2B (B)	$(B - 1) = 1$	$\sigma^2_e + 3\sigma^2_{yab} + 6\sigma^2_{yb} + 9\sigma^2_{ab} + 18\sigma^2_b$
A × B	$(A - 1)(B - 1) = 1$	$\sigma^2_e + 3\sigma^2_{yab} + 9\sigma^2_{ab}$
Y × A	$(Y - 1)(A - 1) = 2$	$\sigma^2_e + 3\sigma^2_{yab} + 6\sigma^2_{ya}$
Y × B	$(Y - 1)(B - 1) = 2$	$\sigma^2_e + 3\sigma^2_{yab} + 6\sigma^2_{yb}$
Y × A × B	$(Y - 1)(A - 1)(B - 1) = 2$	$\sigma^2_e + 3\sigma^2_{yab}$
Error	Total − Model = 271	σ^2_e

3. Results

3.1. Analysis of Variance and Variability

A significant variability in oil and fatty acid composition among the 27 tested varieties was detected (Table 3, Dataset S1). Among all of these traits, the variance of C18:1 (162.64) and C18:2 (116.65) was larger than those of the other traits. The maximum (Max) of C18:1 was 83.02%, while the minimum (Min) of C18:1 was only 42.95%. The distribution of fatty acid composition among different genotypes is shown in Figure 1. Similarly, the Max and Min of C18:2 were 35.01% and 1.59%, respectively. C18:2 had the highest difference (22.02-fold difference) between the Max and Min. C20:0 and C18:0 also had large differences (3.03-fold and 2.46-fold) between the Max (2.18% and 4.60%) and Min (0.72% and 1.87%) than the other traits. According to the variance and standard deviation (SD), several of these traits such as C18:1 and C18:2 could be potentially improved through peanut breeding.

Table 3. Variability in oil percentage on dry weight and fatty acid composition on oil content among selected peanut accessions.

Trait	Mean	Variance	Minimum	Maximum	Max/Mini (fold)	SD
Oil	52.32 (%)	1.89	49.43	56.36	1.14	1.38
C16:0	7.00 (%)	3.28	5.44	11.75	2.16	1.81
C18:0	2.58 (%)	0.70	1.87	4.60	2.46	0.84
C18:1	73.82 (%)	162.64	42.95	83.02	1.93	12.75
C18:2	8.94 (%)	116.65	1.59	35.01	22.02	10.80
C20:0	1.35 (%)	0.09	0.72	2.18	3.03	0.30
C20:1	1.65 (%)	0.18	1.07	1.98	1.85	0.43
C22:0	2.72 (%)	0.21	2.04	3.62	1.77	0.46
C24:0	1.65 (%)	0.06	1.22	1.99	1.63	0.24
C26:0	0.30 (%)	0.01	0.19	0.40	2.11	0.08

SD, standard deviation.

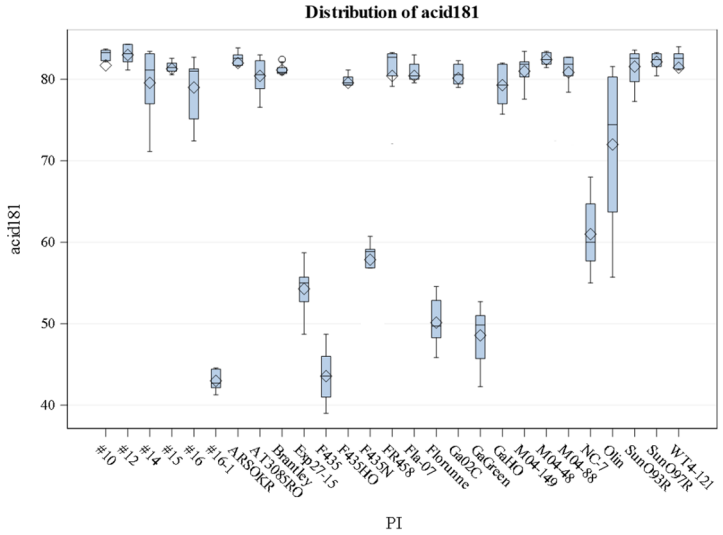

Figure 1. The distribution of fatty acid composition among different genotypes. The unit of the Y-axis is "%".

3.2. Variability in Traits among Accessions, across Locations and Years

The results of statistical analysis of the variable components and their interactions are listed in Table 4. There were significant differences in the variability among the tested varieties (G) for all of the 10 traits. Year also had significant effects on all of the nine fatty acid contents, but not for oil content. No significant differences between replicates for all investigated traits were detected. According to the F values determined through comparisons among the three individual components (year, replicate, genotype), genotype had a significant effect on all of the traits except for C26:0 and C24:0. Year had a larger effect on C26:0 (F value = 102.25 and 96.11, respectively) and C24:0 (F value = 149.87 and 81.69, respectively) than genotype. Among the two-factor interactions (Y × R, Y × G, and R × G), Y × R interaction effects on the variability in C26:0 and C20:0 were significant. Y × G effects and R × G effects on the variability in six traits were significant, but they were not significant for the variability for four other traits (oil content, C16:0, C18:2, C18:1). Interestingly, the three-factor interaction (Y × R × G) effect on the variability was similar to Y × G and R × G effects, which had significant effects on the variability in six traits, but not for four other traits (oil content, C16:0, C18:2, C18:1).

Table 4. Statistical analysis of variable components among 27 tested varieties.

Source	DF	F Value	Pr > F	DF	F Value	Pr > F
		C16:0			Oil	
year (y)	2	12.453	<0.0001	1	0.243	0.6303
rep (r)	2	0.619	0.5411	2	0.838	0.4545
PI (P)	26	125.822	0.0000	25	2.900	0.0244
y ×r	4	0.513	0.7264	2	1.997	0.1752
y × P	47	1.052	0.4148	22	0.889	0.6097
r × P	51	1.472	0.0612	46	0.386	0.9912
y × r × P	77	1.275	0.1437	40	0.421	0.9820
		C26:0			C24:0	
year (y)	2	102.246	<0.0001	2	149.870	<0.0001
rep (r)	2	0.032	0.9686	2	0.394	0.6755
PI (P)	26	96.107	<0.0001	26	81.691	<0.0001
y × r	4	3.868	0.0064	4	1.202	0.3167
y × P	47	5.295	<0.0001	47	8.569	<0.0001
r × P	51	1.791	0.0100	51	4.560	<0.0001
y × r × P	77	2.652	<0.0001	77	3.463	<0.0001
		C22:0			C20:1	
year (y)	2	18.254	<0.0001	2	31.870	<0.0001
rep (r)	2	1.224	0.2997	2	0.684	0.5074
PI (P)	26	79.716	<0.0001	26	164.626	<0.0001
y × r	4	2.147	0.0828	4	3.138	0.0190
y × P	47	7.380	<0.0001	47	5.432	<0.0001
r × P	51	5.798	<0.0001	51	3.282	<0.0001
y × r × P	77	2.882	<0.0001	77	3.930	<0.0001
		C20:0			C18:2	
year (y)	2	15.546	<0.0001	2	8.528	0.0004
rep (r)	2	0.771	0.4659	2	0.747	0.4773
PI(P)	26	100.533	<0.0001	26	116.125	<0.0001
y × r	4	1.347	0.2602	4	0.663	0.6199
y × P	47	3.003	<0.0001	47	0.794	0.8025
r × P	51	2.432	0.0002	51	0.811	0.7863
y × r × P	77	1.976	0.0015	77	0.848	0.7657
		C18:1			C18:0	
year (y)	2	10.794	0.0001	2	39.398	<0.0001
rep (r)	2	0.706	0.4969	2	1.082	0.3440
PI (P)	26	113.710	<0.0001	26	64.923	<0.0001
y × r	4	0.581	0.6775	4	1.501	0.2099
y × P	47	0.815	0.7732	47	1.572	0.0382
r × P	51	0.831	0.7588	51	2.575	0.0001
y × r × P	77	0.806	0.8280	77	1.519	0.0337

3.3. Phenotypic Correlations among Traits

The results of variability in 10 investigated traits among the 27 tested varieties are listed in Table 5. Genotype had significant effects on all of the traits. Table 6 shows Duncan test results on the C18:1 content of four genotypes of *FAD* genes. There were significant effects of both genes *FAD2A* and *FAD2B* on the C18:1 content. The genotype aabb had the highest mean oleic fatty acid contents (80.7%) among the four genotypes (AABB, AAbb, aaBB, aabb). Compared to genotype aaBB, genotype AAbb had a larger effect on the mean oleic fatty acid content. Figure 2 indicates that the fatty acid composition changed with different genotypes of FAD2 alleles. The correlations among the investigated traits are listed in Table 7. The oil content was significantly correlated with C18:0 (r = −0.017) and C24:0 (r = 0.198). The C16:0 content was significantly correlated with all of the tested fatty acid compositions; however, it was negatively correlated with C18:1 (r = −0.969), C20:1 (r = −0.753), C24:0 (r = −0.415), and C26:0 (r = −0.555). The C18:1 content was negatively correlated with C16:0 (r = −0.969), C18:0 (r = −0.408), C18:2 (r = −0.996), C20:0 (r = −0.446), and C22:0 (r = −0.461) and positively correlated with C20:1 (r = 0.737), C24:0 (r = 0.371), and C26:0 (r = 0.511).

Table 5. Comparison of ten investigated traits among 27 tested varieties: oil content is expressed as % of dry weight, and fatty acids as % of oil content.

Variety	Oil (%)	C16:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C20:1 (%)	C20:0 (%)	C22:0 (%)	C24:0 (%)	C26:0 (%)
#10	52.05 bcd	6.05 ghi	2.1 fg	81.73 ab	3.17 g	1.76 fg	1.1 ij	2.08 m	1.65 d–f	0.37 abc
#12	52.26 a–d	5.92 ghijk	2.02 fg	83.02 a	2.13 g	1.79 efg	1.07 j	2.04 m	1.63 d–f	0.38 ab
#14	52.88 ab	6.18 gh	2.3 efg	79.6 ab	4.78 g	1.74 g	1.16 f–j	2.19 lm	1.68 c–f	0.39 ab
#15	52.81 ab	6.26 g	2.35 ef	81.32 ab	2.36 g	1.79 efg	1.27 ef	2.66 f–j	1.71 cde	0.28 gh
#16	52.67 ab	6.33 g	2.53 de	78.92 b	4.54 g	1.75 fg	1.31 de	2.62 g–k	1.71 cde	0.31 fgh
#16-1	50.26 de	11.4 a	3.84 b	42.95 h	35.01 a	0.72 l	1.79 b	2.83 e–h	1.29 ij	0.19 k
ARSOKR	52.62 abc	5.93 ghijk	2.25 efg	82.03 ab	2.26 g	1.93 cdef	1.18 efghij	2.35 kl	1.74 cde	0.33 d–f
AT3085RO	49.43 e	6.02 ghij	2.35 ef	80.35 ab	3.64 g	1.8 d–g	1.24 efgh	2.69 e–j	1.65 d–f	0.27 hi
Brantley	51.17 cd	5.45 jk	4.6 a	81.15 ab	1.59 g	1.21 ij	1.98 a	2.62 g–k	1.22 j	0.19 k
Exp27-1516	53.36 ab	9.55 c	2.77 d	54.29 f	26.3 d	1.13 ij	1.4 cd	2.9 def	1.44 gh	0.22 jk
F435		11.75 a	3.48 bc	43.6 h	33.32 ab	0.91 k	1.69 b	3.62 a	1.43 ghi	0.2 jk
F435HO	53.76 a	7.05 f	2.56 de	79.48 ab	2.4 g	1.75 fg	1.46 c	3.36 bc	1.73 cde	0.24 ij
F435N	56.36 a	9.5 c	3.19 c	57.79 e	21.29 e	1.2 ij	1.71 b	3.54 ab	1.55 fg	0.23 jk
Fla-07	52.48 a–d	6.33 g	2.29 efg	80.37 ab	3.08 g	1.86 c–g	1.26 efg	2.84 e–h	1.71 cde	0.27 hi
Florunner	53.04 ab	10.32 b	2.29 efg	50.15 g	30.08 c	1.23 i	1.28 ef	2.75 e–j	1.62 ef	0.28 h
FR458	52.45 a–d	6.06 ghi	1.91 g	80.36 ab	3.98 g	1.95 cde	1.11 h–j	2.55 h–k	1.75 cde	0.35 b–e
Ga02C	52.62 abc	6.03 ghi	2.28 efg	80.13 ab	2.96 g	2.13 ab	1.25 efg	2.87 efg	1.99 a	0.38 ab
GaGreen	53.15 ab	10.08 b	2.09 fg	48.51 g	31.38 bc	1.46 h	1.22 efghi	3.13 cd	1.84 bc	0.3 fgh
GaHO	52.29 a–d	6 ghijk	3.17 c	79.21 ab	2.86 g	1.74 g	1.7 b	3.45 ab	1.65 d–f	0.22 jk
M04-149	51.87 bcd	5.9 ghijk	1.91 g	80.94 ab	3.55 g	1.99 bcd	1.11 h–j	2.48 jk	1.77 b–e	0.37 a–d
M04-48	51.61 bcd	5.62 ijk	1.97 fg	82.46 ab	2.15 g	1.97 bcde	1.14 g–j	2.54 ijk	1.78 b–e	0.37 a–d
M04-88	52.86 ab	6.15 gh	1.87 g	80.8 ab	3.34 g	2.04 abc	1.09 j	2.58 h–k	1.8 bcd	0.33 c–f
NC-7	52.04 bcd	8.32 d	3.78 b	61.03 d	19.77 e	1.03 jk	1.79 b	2.8 e–i	1.29 hij	0.19 k
Olin	51.11 d	7.63 e	3.63 b	71.97 c	9.27 f	1.2 ij	1.69 b	2.96 de	1.43 ghi	0.22 jk
SunO93R	53.75 a	5.56 ijk	2.08 fg	81.54 ab	2.91 g	1.96 bcde	1.18 efghij	2.58 h–k	1.82 bc	0.38 ab
SunO97R	52.62 abc	5.44 k	1.89 g	82.18 ab	2.31 g	2.18 a	1.1 ij	2.59 g–k	1.91 ab	0.4 a
WT4-121	52.66 ab	6.01 ghijk	1.91 g	81.41 ab	3.16 g	1.93 cdef	1.11 ij	2.47 jk	1.69 c–f	0.32 efg

Note: F435, missing oil data. Means with different letters within the same row are significantly different.

Table 6. Duncan test on oleic fatty acid contents of 4 genotypes of Fad genes.

Fad2a	*Fad2b*	Mean	Std Dev	Significant
AA	BB	43.3	2.64	A
AA	bb	60.8	3.42	C
aa	BB	55.7	6.57	B
aa	bb	80.7	3.12	D

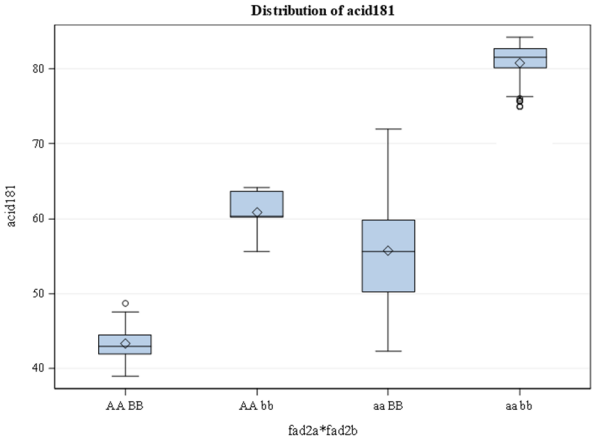

Figure 2. The changes in fatty acid composition with different genotypes of *FAD2* alleles. The unit of the Y-axis is "%".

Table 7. Pearson correlation coefficients and probability values for seed oil content and fatty acid composition among 27 tested varieties.

	Oil	C16:0	C18:0	C18:1	C18:2	C20:0	C20:1	C22:0	C24:0	C26:0
Oil	1	0.133	−0.170 *	−0.131	0.135	−0.149	0.092	0.104	0.198 *	0.083
C16:0		1	0.366 **	−0.969 **	0.962 **	0.396 **	−0.753 **	0.423 **	−0.415 **	−0.555 **
C18:0			1	−0.408 **	0.350 **	0.954 **	−0.737 **	0.371 **	−0.688 **	−0.721 **
C18:1				1	−0.996 **	−0.446 **	0.737 **	−0.461 **	0.371 **	0.511 **
C18:2					1	0.382 **	−0.722 **	0.395 **	−0.365 **	−0.472 **
C20:0						1	−0.713 **	0.560 **	−0.582 **	−0.734 **
C20:1							1	−0.203 **	0.841 **	0.783 **
C22:0								1	0.101	−0.411 **
C24:0									1	0.788 **
C26:0										1

Note: *, significance with $p \leq 0.05$; **, significance with $p \leq 0.01$.

3.4. Additive Effect and Narrow-Sense Heritability of Gene FAD2

The additive effects and the G × E interaction involving two genes (*FAD2A* and *FAD2B*) and three environments (2010, 2011, and 2012) for 27 varieties were calculated (Table 8). The results indicated that the *FAD2B* gene had a larger additive effect (63%) than the *FAD2A* gene (28%). The narrow-sense heritability (h^2) estimate for the *FAD2A* gene was 0.95 ($h^2a = \sigma^2a/[(\sigma^2e/yab) + (\sigma^2yab/yb) + (\sigma^2ya/b) + (\sigma^2ab/y) + \sigma^2a]$; $h^2a = 0.95$) and the narrow-sense heritability (h^2) estimate for the *FAD2B* gene was 0.97 ($h^2b = \sigma^2b/[(\sigma^2e/yab) + (\sigma^2yab/yb) + (\sigma^2yb/b) + (\sigma^2ab/y) + \sigma^2b]$; $h^2b = 0.97$). The additive effect of gene *FAD2B* was larger than that of gene *FAD2A*, which means that peanut breeders can improve high-oleic varieties by editing gene *FAD2B*. No correlation between oil content and *FAD2A* and *FAD2B* genes was found.

Table 8. Additive effects and G × E interaction involving 2 genes (*FAD2A* and *FAD2B*) and 3 environments (2010, 2011, and 2012) for 27 varieties.

Source	Mean Square	F Value	Pr > F	σ^2	Additive Effect and Interaction
Year (Y)	31.98	2.18	0.1153		
Rep (year)	5.2	0.36	0.9057		
FAD2A (A)	2495.2	169.87	<0.0001	130.67	0.28
FAD2B (B)	5468.9	372.32	<0.0001	292.42	0.63
A×B	167.4	11.40	0.0008	15.88	0.03
Y×A	0.26	0.02	0.8936		
Y×B	62.5	4.25	0.0152	6.33	0.01
Y×A×B	24.5	1.67	0.1973	3.27	0.01
Error	14.68			14.68	0.03

3.5. AMMI1 Biplot Display

The AMMI analysis was utilized to quantify the impact of the environment on the genotypes. Biplot analysis is a valuable tool for interpreting AMMI models. Two types of AMMI biplots were generated: the AMMI1 biplot, which displayed the interaction between the genotype mean and environments; and the AMMI2 biplot (GGE biplot), which showed scores for IPCA1 and IPCA2 [26]. From Figure 3, it is evident that Env1 had the greatest main effects and was favorable for the performance of most genotypes. Conversely, Env3 exhibited lower main-effect values, indicating little interaction with genotypes. Env2 had a positive PC score with a high mean value. Genotypes #12, M04-48, and SunO97R were identified as well adapted to both Env2 and Env3, suggesting these two environments as suitable for these three genotypes. Genotypes F435, Florunner, Exp27-1516, Ga02C, ARSOKR, SunO97R, #12, M04-48, and GaHO displayed PC1 scores close to zero, while other genotypes demonstrated a below-average oleic acid content with negative PC scores or an above-average oleic acid content with positive PC scores. Genotypes #16-1 and F435 had a lower C18:1 content, whereas genotypes #12, M04-48, and SunO97R had a higher C18:1 content. Moreover, Env1 had a large negative PC1 score, which positively interacted with genotypes with negative PC1 scores like Olin, and negatively interacted with genotypes with positive PC1 scores. Finally, the AMMI1 biplot statistical model was employed to identify G × E interactions in peanut. Genotypes #10, #12, ARSOKR, AT3085RO, Brantley, Ga02C, GaHO, M04-48, and SunO97R were suitable for planting in Env2 and Env3, while genotypes #15, #16, F435HO, Fla-07, FR458, M04-149, M04-88, SunO93R, and WT4-121 were considered favorable environments for Env1.

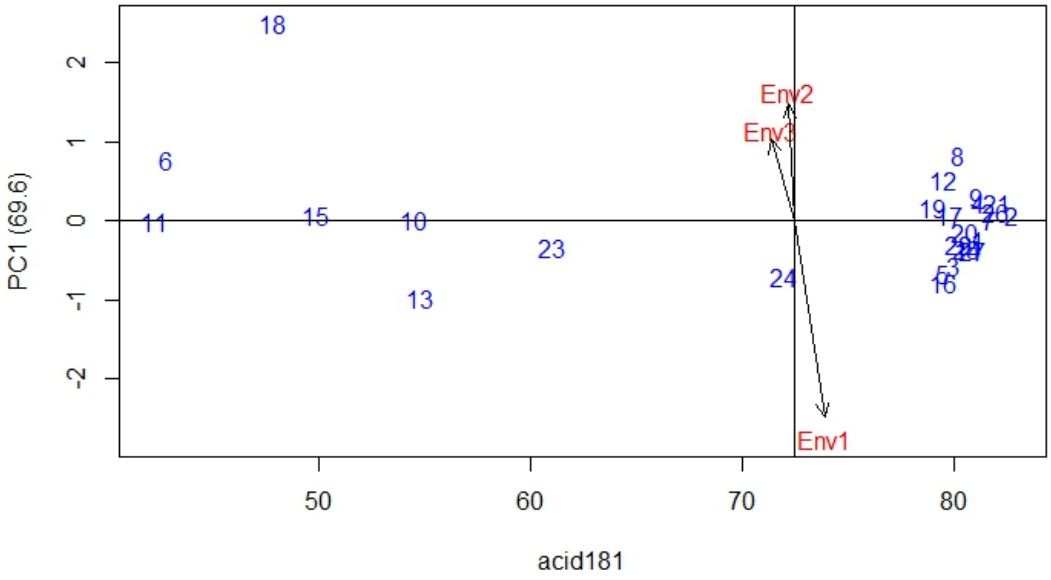

Figure 3. AMMI1 biplot for oleic acid of 27 genotypes tested in three environments. The three environments are Env1, GA in 2010; Env2, AL in 2011; and Env3, AL in 2012. A total of 27 genotypes are represented as #10, #12, #14, #15, #16, #16-1, ARSOKR, AT3085RO, Brantley, Exp27-1516, F435, F435HO, F435N, FR458, Fla-07, Florunner, Ga02C, GaGreen, GaHO, M04-0149, M04-048, M04-088, NC-7, Olin, SunOleic93R, SunOleic97R, WT4-121 in order. PC, principal component.

3.6. AMMI2 Biplot Display

To assess the environment cluster differentiation, genotype-specific adaptation, and G × E interaction, a biplot illustrating the performance of 27 genotypes in three environments was generated (Figure 4). In Figure 4, the environments were categorized into three sections. Among them, Env3 exhibited short spokes and demonstrated weak interactive forces, whereas Env1 and Env2 displayed long spokes, indicating their discriminatory nature. In the AMMI 2 biplot, the genotype GaGreen was more responsive since it was

more distant from the origin and suitable for Env2 and Env3. With the exception of #10, #14, #16-1, AT3085RO, F435N, GaGreen, and Olin, most genotypes were located near the origin, implying their lower sensitivity to environmental interactive forces. Overall, based on the findings from AMMI1 and AMMI2, genotypes #12, #15, ARSOKR, Brantley, GaHO, M04-149, M04-48, and SunO97R were identified as the best performers in terms of high oleic contents, making them favorable for Env3.

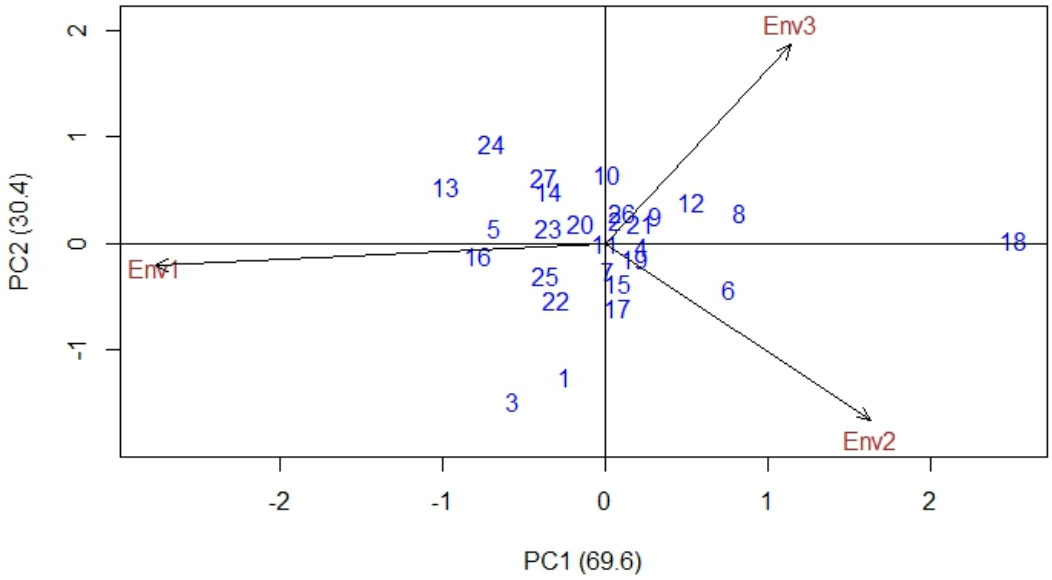

Figure 4. AMMI2 GGE biplot representing a vector view of the three environments used to test the mean oleic acid of 27 peanut genotypes. The three environments are Env1, GA in 2010; Env2, AL in 2011; and Env3, AL in 2012. A total of 27 genotypes are represented as #10, #12, #14, #15, #16, #16-1, ARSOKR, AT3085RO, Brantley, Exp27-1516, F435, F435HO, F435N, FR458, Fla-07, Florunner, Ga02C, GaGreen, GaHO, M04-0149, M04-048, M04-088, NC-7, Olin, SunOleic93R, SunOleic97R, WT4-121 in order. PC, principal component.

4. Discussion

A correlation between oleic acid content and *FAD2A* and *FAD2B* genes has clearly been demonstrated by many researches; however, the relative contribution of *FAD2* genes (*FAD2A* and *FAD2B*) to oleic acid quality traits in peanut is still unknown [27–30]. The mutant allele *FAD2A* is widely available in the U.S. peanut germplasm collection but the mutant allele *FAD2B* is only present in the selected genotypes such as SunOleic 95R and SunOleic 97R [31,32]. There are no studies on estimating the contribution of these two mutant alleles to oleic acid and we do not know which of the mutant alleles could produce more oleic acid [32]. Researchers have just examined how the interactions of additive alleles determine the content of oleic acid; however, each allele can have an additive effect as well. The additive effects of two alleles were calculated for improving the further understanding of the genetic control for oleic acid synthesis in peanut.

The AMMI analysis is a most popular method to study G×E interactions and it was used to quantify the effect of G, E, and G × E interactions on drought-related traits in peanut [33]. In addition, the effectiveness of the AMMI procedure has been clearly demonstrated in other crops, such as wheat [34], soybean [35], maize [36], pear millet [37], and field pea [38]. However, it still has its own limits; for example, it does not provide a measure for quantitative stability [39]. In this study, 27 peanut genotypes were evaluated in a three-year (2010–2012) field experiment, which was conducted at two locations. The combination of the AMMI model and biplot made it possible to describe the genotype-by-environment interactions effect more accurately.

Compared to regular peanuts, the high-oleic peanuts are more naturally resistant to oxidation because it is higher in monounsaturated fats. The peanut single kernel oleic

acid distributions were influenced by seed size, seed maturity, growing environment, and season flower termination [40]. The main chemical technique is gas chromatography (GC), which calls for 100 extractions or injections of a standard sample and an experienced operator. Although more affordable and quicker than the GC technique, refractive index approaches still need a significant amount of time to complete [41]. However, they exhibit a good correlation with the principal GC method. Numerous breeding efforts are using near-infrared (NIR) techniques to measure this fatty acid chemistry. It is also critical to understand how costly and time-consuming it is to measure this chemistry. Therefore, it is better to select varieties by genotype with higher additive effects and it is easy to obtain the genetic gain. For companies, quality is important, so they need to consider where to grow these high-oleic peanuts. The results from this study could help breeders and companies to obtain peanuts with stable high oleic contents.

5. Conclusions

In the present study, high-oleic and stable genotypes, such as genotypes #12, #15, ARSOKR, Brantley, GaHO, M04-149, M04-48, and SunO97R, could be used as new potential genetic resources for improving the peanut varieties with contents of oleic. In addition, the results also indicated that the *FAD2B* gene had a larger additive effect than the *FAD2A* gene, which provides important values for breeding high-oleic peanut varieties by editing gene *FAD2B*.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae9121272/s1, Dataset S1: The phenotype and genotype data of varieties used in this study.

Author Contributions: H.Z., Y.Y., and C.C. designed the study and performed the data analysis; M.W. and P.D. contributed to sample preparation and chemical analysis; H.Z., Y.Y., and C.C. wrote the draft manuscript. All authors contributed to the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This project was supported by "Double first-class discipline promotion project" of South China Agricultural University (grant number 2021B10564001), the Guangzhou science and technology planning project (grant number 2023A04J1449) and The Peanut Foundation (grant number 04-833-17).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the supplementary material.

Acknowledgments: We are indebted to Sam Hilton, Brian Gamble, Larry Wells, and Jason Burkett for their devoted assistance with the management of field experimental plots at the Wiregrass Research and Extension Center and the E.V. Smith Research Center. Thanks also to Joseph Powell, and other personnel from the USDA-ARS National Peanut Research Lab at Dawson, GA, for assistance with the experimental aspects associated with this research.

Conflicts of Interest: Authors declare that there are no conflict of interest.

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