Seed Composition Survey of a Peanut CSSL Population Reveals Introgression Lines with Elevated Oleic/Linoleic Profiles

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ABSTRACT

The peanut CSSL population represents one of the ways that interspecific hybridization has been used to introduce genetic variation into cultivated peanut. The lines were developed by crossing Fleur 11, a farmer preferred spanish cultivar from West Africa with a synthetic allotetraploid. The latter was developed by crossing *A. duranensis* to *A. ipaensis* and tetraploidizing the resultant hybrid. Subsequent selection with genetic markers resulted in a population comprising lines with small chromosome segments from the wild in a cultivated peanut background. The objective of this study was to characterize the protein, total oil, fatty acid and sugar profiles of the population. The results indicated that the values of Fleur 11 for all the traits analyzed were within the normal range expected in peanut. Since the population had a uniform genetic background derived from Fleur 11, the profiles for a majority of the lines were comparable to Fleur 11. However, three lines (CSSL 84, CSSL 100 and CSSL 111) were found to have elevated oleic acid and reduced linoleic and palmitic acid relative to Fleur 11. The oleic to linoleic acid ratios (O/L) for these lines were 118, 104 and 97% greater than that of Fleur 11, respectively. While the increased values are still considered to be within the normal oleic acid range, the effect of introgressions on these lines represent the possibility of discovering new sources of high O/L polymorphisms. Such polymorphisms have the potential for use in further improving peanut oil quality.

Key Words: Fatty acid, Fleur 11, protein, sugar profiles, synthetic allotetraploid.

The global population is on a steady increase with the expectation that it will peak at approximately 9.5 billion people by 2050 (Godfray *et al.*, 2010). This imposes a major challenge of ensuring food supply in sufficient quantities and quality. The problem is compounded by the effects of climate change and economic as well as political volatility that have stymied progress towards achieving food security (Lobell *et al.*, 2011; Dawson *et al.*, 2016). In the past, addressing these challenges involved intensifying agricultural production with the aim of meeting calorie demands as exemplified in the green revolution (Pingali, 2012; Mehta, 2018). However, it is increasingly recognized that ensuring food security necessitates looking beyond hunger and facilitating access to nutritious food in adequate quantities (FAO, 2019).

The nutritional profile of peanut makes it an excellent source of both caloric components and micronutrients that exert a positive contribution to human health. The peanut seed is composed of 41.2 to 58.6% oil, 12 to 36% protein (Savage and Keenan, 1994) as well as a host of other beneficial micronutrients such as tocopherols, folates, flavonoids, phenolics and free amino acids (Francisco and Resurreccion, 2008). The compendium of nutrients and bioactive compounds makes peanut a useful nutraceutical (Akram *et al.*, 2018; Toomer, 2018). In this regard, it has been extensively used as a ready to use therapeutic food (RUTF) in treating and reversing the effects of severe acute malnutrition especially in children (Bailey, 1963; Enserink, 2008; Israëls *et al.*, 2009). It is also used as a ready to use supplementary food (RUSF) in managing early stage malnutrition (Variath and Janila, 2017).

The oil profile of the legume contains a proportionally favorable composition of fatty acids. Up to 90% of the fatty acid content is comprised of palmitic, oleic and linoleic acid, with oleic and linoleic acid making up 80% of this composition (Norden *et al.*, 1987; Dean *et al.*, 2009). In high oleic peanut cultivars, the ratio of oleic acid to linoleic acid (O/L) makes peanut an ideal source of monounsaturated fatty acids, which have been shown to have good health benefits. These include reduction of hypertensive effects (Terés *et al.*, 2008), anti-inflammatory effects (Carrillo *et al.*, 2012) as well as alleviation of type II diabetes and reversal of negative effects of obesity (Vassiliou *et al.*, 2009). In addition, the high

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oleic peanut has been demonstrated to reduce the levels of serum cholesterol and low-density lipoprotein cholesterol, resulting in improvement of serum lipid and apolipoprotein levels in postmenopausal women (O'Byrne *et al*., 1997). When used as feed, high oleic peanut increases the amount of monounsaturated fat by up to 32% while reducing the amount of polyunsaturated fat in pigs (Myer *et al*., 1992). This indirectly contributes to human nutrition. Aside from its health consequences, this oil composition confers oxidative stability, with a net positive effect on the quality of its products (O'Keefe *et al*., 1993).

China produced 36% of global peanut in 2017, followed by India, US and Nigeria each of which accounted for 19, 7 and 5% of global production, respectively (FAOSTAT, 2017). In China and India, most of the peanut produced is crushed for oil extraction with high oil content varieties being preferred. In the US the main use is confection and other food purposes with the preference being for lower oil content. In Africa, food and oil use of peanut is comparable (Birthal *et al*., 2010; FAOSTAT, 2014; Janila *et al*., 2016a; 2016b). This varied use of the legume underscores the need for varieties with both good nutritive and oil profiles.

Since the discovery of two high oleic lines (F435-2-1 and F435-2-2) with 80% oleic and 2% linoleic acid (Norden *et al*., 1987), incorporating the high O/L trait in breeding lines has become integrated in the breeding programs of the US. This trait results from the action of two non-allelic homeologous genes, *ahFAD2A* and *ahFAD2B,* with 99% sequence homology. Inactivation of these genes results in absence of Δ^{12}-desaturase enzyme activity that catalyzes conversion of oleic acid to linoleic acid (Jung *et al*., 2000a; 2000b). In the US, development of high O/L cultivars was accelerated by implementing the molecular marker for the mutant allele of *ahFAD2B* in breeding programs, enabling the pyramiding of the high O/L trait with other traits of interests (Chu *et al*., 2009,2011). Consequently, marker assisted selection is routinely implemented to pyramid the high O/L trait with other traits of interest (Chu *et al*., 2011). Other peanut producing countries such as India, China, Brazil, Argentina, South Africa, Israel, Japan and Australia are also including the high O/L trait in their peanut (Janila *et al*., 2016b; Nawade *et al*., 2018). However, information on studies of oil and other seed composition traits from West African lines is seldom available.

In this study, we analyzed the seed composition profile of a chromosome segment substitution line (CSSL) population. The cultivated parent used to create the population was Fleur 11, an important spanish type peanut cultivar grown in Senegal, West Africa. In a previous study, Fonceka *et al*. (2012) crossed this variety with a synthetic allotetraploid peanut derived from the diploid progenitors of cultivated peanut (Fávero *et al*., 2006). A total of 122 lines were developed, each of which contained a small chromosomal segment from the wild in such a way that the entire wild genome was represented in the whole CSSL population (Fonceka *et al*., 2012). Since the CSSL population has a uniform genetic background, it was hypothesized that phenotypic variations that distinguish the lines from Fleur 11 would be due to presence of wild introgressions. With that in mind, our objective was to investigate the effects of these introgressions on oil content, fatty acid composition, sugar profile and protein content on a subset of the CSSLs relative to Fleur 11.

Materials and Methods

The composition profiles of 77 CSSLs were assayed in the growing seasons of 2016 and 2017. The parental check Fleur 11, was included in both years while two other cultivated checks, OLin (Simpson *et al*., 2003) and New Mexico Valencia A (Hsi and Finkner, 1972), were planted in the second year. The plants were grown in the field at the University of Georgia (Tifton) Gibbs farm (31°26′04.7" N 83°35′18.4" W). The soil type was Tift loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults), and the trial plan was a randomized complete block design (RCBD) with three replicates in each year. Each two-row plot was 3 m long with the seeds planted at a spacing of 5 cm. Conventional agronomic practices included scheduled fungicide and pesticide applications as well as regular irrigation as required. At the end of the season, seeds for the population were harvested, dried and inspected for maturity as indicated by darkening of the endocarp. From each line, 5 grams of fully mature seed were collected, packaged in a labeled freezer bag and shipped to the USDA ARS Market Quality and Handling Research unit (MQHRU) lab at the North Carolina State University. Assays for fatty acid profile, sugar profile, total fat and total protein were conducted as follows.

Total protein was determined using a Sprint Titrator (CEM, Matthews, NC). This instrument uses a proprietary solution to tag the amine side chains of proteins. The resulting solution is fed through a visible light detector that converts the signal to percent protein. The instrument was factory calibrated for peanuts.

Total sugars were determined using the method first described by Pattee *et al*. (2000a). Mono, di and trisaccharides were extracted from the samples after defatting with hexane. A mixture of chloroform, methanol and water (60/25/15 v/v/v) was used. The organic solvents were removed from the extracts by evaporation under nitrogen. The remaining aqueous solution was spiked with internal standard solution (lactose and cellobiose, Sigma Chemical Corp., St. Louis, MO). Finally, 50 μL of each sample solution was diluted to 2 mL with water, passed through a Dionex OnGuard-H filter (Dionex, Sunnyvale, CA), and injected onto a Dionex Bio LC system. A Dionex CarboPac™ PA-1 column (250 mm length, 4 mm i.d.) and a Pulsed Amperometric Detector (PAD) was used. The column was heated to 25°C. The mobile phase was 200 mM sodium hydroxide at a flow rate of 1.0 mL/min. The sugars present were quantified using response ratios to the internal standards compared to those of authentic standards of myo-inositol, glucose, fructose, sucrose, raffinose, and stachyose (Sigma).

The total fat in the samples was determined using a Mini Spec Seed Analyzer, (Bruker Instruments, Billerica, MA). Five grams of sample were loaded into a glass sample tube and inserted into the instrument. This instrument uses time domain or low power nuclear magnetic resonance. It is used for total fat determination in oilseeds according to AOCS method, AOCS Ak4-95. The instrument was calibrated daily using a sealed tube of standard reference canola seed as instructed by the instrument manual. A standard curve of weighed amounts of peanut oil spiked into an inert matrix was run weekly. The variability on repeated analyses was less than or equal to 0.01%.

For fatty acid profile determination, oil was expressed from the samples using a hydraulic press (Fred Carver and Assoc., Wabash, IN). After saponification to release the fatty acids from the triglycerides, the fatty acids in the oil were methylated using a boron-trifluoride catalyst (Bannon *et al*., 1982). The resulting methyl esters were analyzed using gas chromatography with flame ionization detection (PE Autosampler XL, Perkin Elmer Instruments, Shelton, CN) and quantified as the percent of each fatty acid present of all the fatty acids identified according to the AOCS official method (2004) # Ce 1f-96. Authentic fatty acid methyl esters (Kel Fir FAME 5 mixture, Matreya, LLC, State College, PA) were used for identification by retention times.

Data received were analyzed using R (R Core Team, 2013). Normality of the data was tested using the Shapiro-Wilk test and data that did not follow a normal distribution was subjected to BoxCox analysis to determine appropriate transformations to bring the traits to approximate normality (Box and Cox, 1964). Partitioning of variance was done using mixed model linear regression with the LmerTest package (Kuznetsova *et al*., 2017) with FDR used for multiple hypothesis correction. Trait summaries were derived from the obtained coefficients. For statistically significant traits ($P < 0.05$), lines that were significantly different from Fleur 11 ($P < 0.05$) were determined by running a Dunnett's multi-comparison test (Dunnett, 1955). The relative effects of introgression on the traits were calculated by taking the difference between the coefficients of each line and Fleur 11 and getting the percentage relative to Fleur 11. PCA biplot analysis was done to examine correlations between the traits that exhibited significant differences among the lines.

Results and Discussion

The spontaneous tetraploidization event that originated cultivated peanut also introduced a reproductive barrier that prevented genetic exchanges with wild diploid relatives. The resultant bottleneck reduced genetic diversity of cultivated varieties. The tetraploid route to interspecific hybridization recently has played a vital role in bridging the ploidy gap and accessing useful alleles from the wild (Simpson, 2001; Fávero *et al*., 2006). CSSLs, which were developed by the judicious selection of small chromosome segments from the wild in a cultivated background provide an important resource for characterizing the effects of wild alleles on phenotype (Fonceka *et al*., 2012). In this study, we examined whether wild alleles have an impact on seed quality attributes of a subset of the CSSL population comprising 77 lines that represented approximately 78% of the wild peanut genome. These attributes included 17 fatty acid profiles (palmitoleic, palmitic, margaric, heptadecenoic, stearic, oleic, linoleic, g-linoleic, arachidic, eicosenoic, eicosadienoic, behenic, erucic, lignoceric, cerotic and other acids as well as iodine value), seven sugar profiles (fructose, glucose, myo-inositol, raffinose, stachyose, sucrose and total sugars), O/L ratio, total oil, and protein.

The composition profile of peanut is important as its various physico-chemical constituents contribute to the nutritional, taste, flavor and textural quality of the legume and its products (Ahmed and Ali, 1986; Dwivedi *et al*., 2000). These traits have a genetic basis, but they also vary depending on environmental factors such as year and area of

cultivation as well as the interactions of genetics and environment (Branch *et al*., 1990; Ku *et al*., 1998; Andersen and Gorbet, 2002). In line with this, Isleib *et al*. (2008) in a study of seed composition traits of various breeding lines noted that genetic variation was appreciably high for fatty acid traits in contrast to oil content and sugar profiles, which varied largely as a result of environment. This is consistent with our observation of lower genetic variance for sugar traits compared to the oil components (Table 1).

For instance, all but two sugar traits (myo-inositol and stachyose) had no significant genetic differences in 2016 in contrast to 2017 where genetic differences were observed for all traits. This lack of stability across years shows that these sugar profile differences are a product of interactions between genetic and environmental factors rather than just the effects of introgressions. In terms of quantity, sucrose was the most abundant sugar while fructose was least abundant in both years (Table 1). Sucrose contributes to the sweetness attribute of both roasted and unroasted peanut. Upon roasting, reducing sugars are liberated from sucrose, which interact with various amino acids to produce the characteristic nutty flavor associated with roasted peanut (Newell *et al*., 1967; Mason *et al*., 1969; Pattee *et al*., 2000b).

Comparison of the sugars with Fleur 11 identified two lines (CSSL 32 and CSSL 53) that had increased fructose in 2016. CSSL 84 had increased myo-inositol in 2016, CSSL 111 and CSSL 84 had increased raffinose in 2017 and 14 other lines had reduced stachyose in 2017 (Supplementary Table 1). These results do not replicate across the years and are likely due to interaction of the introgressions with environmental factors. In an ideal peanut, the desirable sugar profile would be characterized by high sucrose because of its flavor enhancing properties and reduced raffinose and stachyose (Bishi *et al*., 2015). Raffinose and stachyose belong to the raffinose family of oligosaccharides (RFOs) which are undesirable since they are not digested by humans due to lack of the α-GAL enzyme. Instead, they are fermented in the large intestines by bacteria resulting in production of hydrogen, carbon dioxide and methane leading to discomforts associated with gassiness (Bryant *et al*., 2003; Tahir *et al*., 2012). These sugars are inconsequential for oil production, but significant in food use (Bishi *et al*., 2013). It would be beneficial to identify lines with genetic variation that increase sucrose and reduce the RFOs.

The mean total oil and protein contents of the CSSLs was 52 and 27% respectively. These values are within the normal range for cultivated peanut (Savage and Keenan, 1994; Grosso and Guzman, 1995; Young and Tai, 2010). While there was overall significant difference among the lines for oil in 2017 and protein in both years (Table 1), none of the individual lines had statistically significant difference from Fleur 11 for both traits. This shows that the introgressions in this population had minimum impact on oil and protein content.

Palmitic, oleic and linoleic acids make up 90% of all fatty acids in peanut. Of these, palmitic acid takes up 10% of the proportion while the unsaturated oleic and linoleic acids make up the remaining 80%. Stearic, lignoceric, behenic, arachidic and eicosenoic acid comprise between 0.02-4.0% of total fatty acids with the rest of the proportion accounted for by other fatty acids (Andersen and Gorbet, 2002). Generally, unsaturated fatty acids can be oxidized resulting in aldehydes, ketones, acids and hydrocarbons that are responsible for diminishing the shelf life and nutritional quality of peanut (Moore and Knauft, 1989; Andersen and Gorbet, 2002). The degree of fatty acid unsaturation results in the classification of the fatty acids as monounsaturated or polyunsaturated, denoting the presence of one or multiple double bonds in the fatty acid side chain respectively. Monounsaturated fatty acids exhibit less oxidation than polyunsaturated fatty acids and are more preferred. Exponential increase in oxidative stability with increasing O/L has been demonstrated (O'Keefe *et al*., 1993; Davis *et al*., 2016). Hence, the ratio of the monounsaturated omega 9 oleic acid to the polyunsaturated linoleic acid is a critical quality parameter in peanut. In addition to the quality enhancing property of the trait, many health benefits have also been attributed to this trait including hypotensive and anti-inflammatory effects as well as reduction of type II diabetes and obesity (Terés *et al*., 2008; Vassiliou *et al*., 2009; Carrillo *et al*., 2012).

Oleic, linoleic, palmitic, stearic, arachidic and behenic acid are known to have a strong genetic component (Isleib *et al*., 2008). The mean values observed in this study (46.7% for oleic, 32.3% for linoleic, 10.2% for palmitic, 4.4% for stearic, 1.7% for arachidic and 2.7% for behenic) (Table 1), were within ranges observed in other studies (Worthington *et al*., 1972). Oleic acid was inversely related to linoleic, palmitic, behenic, arachidic and stearic acid, with a positive correlation observed with eicosenoic acid. The association of oleic acid with iodine value (a measure of degree of oil unsaturation and hence a measure of oil stability) was inverse (Figure 1). These relationships observed were in general agreement with those of other studies (Mozingo *et al*., 1989; Hashim *et al*., 1993;

Table 1. Statistical summary of the chromosome segment substitution lines' seed composition traits. The lines were developed by introgressing chromosome segments of a synthetic allotetraploid into the background of a cultivated spanish variety of peanut (Fleur 11).

Year	Traits	Unit	Significance	Mean	Fleur 11[a]	Min[b]	Max[c]	Sdev[d]	SE[e]	Heritability[f]
2017	Palmitic	%FA[g]	S***	10.072	12.598	8.336	12.598	0.644	0.073	0.506
2016	Palmitic	%FA[g]	S***	10.247	12.309	8.881	12.309	0.583	0.066	0.438
2017	Palmitoleic	%FA[g]	S**	0.031	0.040	0.000	0.072	0.018	0.002	0.139
2016	Palmitoleic	%FA[g]	NS	0.019	0.056	-0.001	0.058	0.014	0.002	0.104
2017	Margaric	%FA[g]	S***	0.029	<0.001	<0.001	0.099	0.019	0.002	0.265
2016	Margaric	%FA[g]	NS	0.047	0.066	0.004	0.123	0.018	0.002	0.082
2017	Heptadecenoic	%FA[g]	S***	0.003	<0.001	<0.001	0.037	0.006	0.001	0.244
2016	Heptadecenoic	%FA[g]	S***	0.005	0.020	-0.003	0.033	0.007	0.001	0.228
2017	Stearic	%FA[g]	S***	5.206	3.240	2.799	7.529	0.899	0.102	0.425
2016	Stearic	%FA[g]	S***	3.555	2.727	2.239	5.450	0.530	0.060	0.389
2017	Oleic	%FA[g]	S***	45.803	44.559	39.756	60.973	3.695	0.421	0.737
2016	Oleic	%FA[g]	S***	47.741	45.809	41.670	62.111	3.321	0.376	0.547
2017	Linoleic	%FA[g]	S***	31.887	33.728	20.829	35.677	2.694	0.307	0.690
2016	Linoleic	%FA[g]	S***	32.730	33.491	20.914	37.370	2.684	0.304	0.563
2017	G-linoleic	%FA[g]	S**	0.022	<0.001	<0.001	0.058	0.015	0.002	0.167
2016	G-linoleic	%FA[g]	NS	0.008	0.013	-0.001	0.031	0.006	0.001	0.051
2016	Arachidic	%FA[g]	S***	1.479	1.232	1.100	1.952	0.137	0.016	0.517
2017	Arachidic	%FA[g]	S***	1.964	1.359	1.254	2.446	0.234	0.027	0.403
2017	Eicosenoic	%FA[g]	S***	0.744	0.764	0.511	1.154	0.112	0.013	0.547
2016	Eicosenoic	%FA[g]	S***	0.614	0.659	0.472	0.979	0.081	0.009	0.390
2017	Behenic	%FA[g]	S***	2.984	2.591	2.070	3.740	0.341	0.039	0.328
2016	Behenic	%FA[g]	S***	2.484	2.428	2.057	2.964	0.175	0.020	0.278
2017	Erucic	%FA[g]	S***	0.031	<0.001	<0.001	0.089	0.024	0.003	0.180
2016	Erucic	%FA[g]	NS	<0.001	<0.001	<0.001	0.008	0.001	0.000	0.000
2016	Lignoceric	%FA[g]	S***	0.919	0.991	0.751	1.226	0.091	0.010	0.274
2017	Lignoceric	%FA[g]	S***	1.109	1.041	0.842	1.594	0.142	0.016	0.273
2016	Cerotic	%FA[g]	S***	0.116	0.123	0.041	0.181	0.026	0.003	0.317
2017	Cerotic	%FA[g]	S***	0.123	0.079	0.010	2.079	0.228	0.026	0.095
2017	Eicosadienoic	%FA[g]	NS	0.004	<0.001	<0.001	0.016	0.004	0.001	0.015
2017	O/L	ratio	S***	1.475	1.322	1.127	2.989	0.332	0.038	0.677
2016	O/L	ratio	S***	1.492	1.376	1.132	2.990	0.282	0.032	0.594
2016	Iodine		S***	97.751	97.413	89.649	101.016	1.928	0.218	0.526
2017	Iodine		S***	94.648	96.746	87.663	97.531	1.749	0.199	0.511
2017	Oil	%w/w[h]	S***	52.498	55.617	48.846	55.617	1.305	0.149	0.307
2016	Oil	%w/w[h]	NS	52.950	52.668	50.158	56.216	1.181	0.134	0.062
2016	Myo-inositol	mg/g	S*	156.888	164.404	119.158	381.442	32.299	3.657	0.353
2017	Myo-inositol	mg/g	S**	217.728	280.268	144.143	314.869	37.268	4.247	0.131
2017	Glucose	mg/g	S**	28.049	29.256	14.980	54.464	7.570	0.863	0.088
2016	Glucose	mg/g	NS	55.757	53.118	40.001	92.650	10.953	1.240	0.031
2017	Fructose	mg/g	S***	25.688	27.100	10.143	42.353	8.048	0.917	0.160
2016	Fructose	mg/g	NS	48.779	34.369	26.536	98.940	13.490	1.527	0.129
2017	Raffinose	mg/g	S***	607.657	597.083	363.417	1281.870	140.352	15.995	0.348
2016	Raffinose	mg/g	NS	376.202	361.769	248.516	688.268	82.188	9.306	0.044
2017	Stachyose	mg/g	S***	2934.247	4240.040	1295.437	4880.289	766.324	87.331	0.331
2016	Stachyose	mg/g	S**	1522.062	1937.130	1027.503	2680.488	329.737	37.335	0.209
2017	Sucrose	mg/g	S*	32,272.950	30,543.331	26,441.818	40,798.069	2885.492	328.832	0.158
2016	Sucrose	mg/g	NS	17,666.842	15,757.071	13,721.039	21,021.487	1724.957	195.313	0.076
2017	Total Sugars	mg/g	S*	36,086.095	35,717.081	28,823.392	45,204.719	3401.275	387.611	0.156
2016	Total Sugars	mg/g	NS	19,832.893	18,307.860	15,458.784	23,993.851	1896.759	214.766	0.098
2016	Protein	g/100g	S***	27.552	27.637	24.396	30.156	1.285	0.146	0.213
2017	Protein	g/100g	S*	26.476	23.689	22.463	29.532	1.209	0.138	0.110

Table 1. Continued.

[a]Cultivated background spanish variety parent of the CSSL population
[b]Minimum
[c]Maximum
[d]Standard deviation
[e]Standard error
[f]Heritability was estimated in the broad sense
[g]Percent fatty acid
[h]Percent weight by weight
NS: Not significant
S* Significant at $P < 0.05$
S** Significant at $P < 0.01$
S*** Significant at $P < 0.001$

Andersen *et al*., 1998). Overall, oleic, linoleic and palmitic acid as well as, O/L were the most heritable traits with the heritabilities similar in both years (Table 1). Of the 210 line by trait differences consistent in both years (Table 2), the most represented traits in this comparison were reduction of palmitic acid, increase in arachidic acid and increase in stearic acid with 68, 16 and 5 comparisons, respectively. The lines with the highest number of trait comparisons were CSSL 84, CSSL 100 and CSSL 111, each having 6 comparisons (Table 2). In both years, these lines exhibited reduction in palmitic acid, linoleic acid, and increase in oleic acid, eicosenoic acid and O/L.

The O/L depends on individual concentrations of oleic and linoleic acid. However, the effect of O/L on other fatty acids such as palmitic, total C18 and total saturated fatty acids has also been observed, suggesting a pleiotropic effect. This is in addition to epistatic relationships of the same

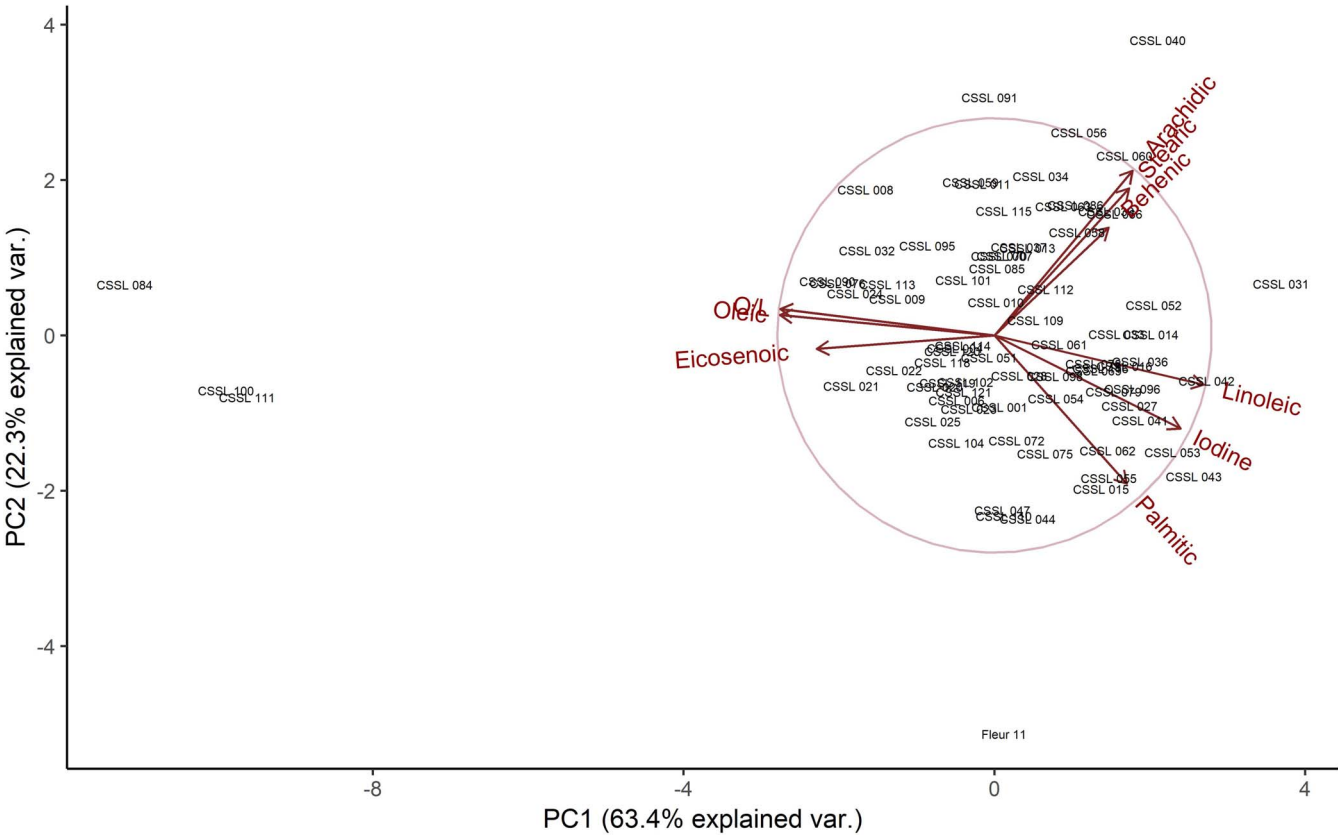

Fig. 1. PCA biplot showing the relationships between fatty acids that had significant differences among the chromosome segment substitution lines (CSSLs) in 2016 and 2017. Oleic acid (and hence the O/L) is shown to have an inverse relationship with linoleic, palmitic, behenic, arachidic and stearic acid as well as iodine value. It is positively related to eicosenoic acid. Also observed is a clear separation of CSSL 84, CSSL 100 and CSSL 111 from the rest of the population.

Table 2. Effects of introgressions relative to Fleur 11 for traits with consistent significant difference in both 2016 and 2017.

Sample	Trait	Year	Effect (%)	Sample	Trait	Year	Effect (%)
CSSL 001	Palmitic	2016	-16.682	CSSL 066	Stearic	2017	80.041
CSSL 001	Palmitic	2017	-20.012	CSSL 066	Arachidic	2017	68.52
CSSL 004	Palmitic	2016	-11.699	CSSL 066	Palmitic	2017	-22.57
CSSL 004	Palmitic	2017	-17.101	CSSL 069	Palmitic	2016	-12.8
CSSL 007	Palmitic	2016	-23.019	CSSL 069	Palmitic	2017	-16.476
CSSL 007	Palmitic	2017	-20.489	CSSL 070	Palmitic	2016	-17.548
CSSL 008	Palmitic	2016	-24.4	CSSL 070	Palmitic	2017	-23.523
CSSL 008	Palmitic	2017	-27.289	CSSL 072	Palmitic	2016	-14.353
CSSL 009	Palmitic	2016	-18.303	CSSL 072	Palmitic	2017	-19.73
CSSL 009	Palmitic	2017	-24.202	CSSL 075	Palmitic	2016	-13.477
CSSL 010	Palmitic	2016	-19.451	CSSL 075	Palmitic	2017	-15.144
CSSL 010	Palmitic	2017	-23.84	CSSL 076	Palmitic	2016	-19.3
CSSL 011	Palmitic	2016	-18.857	CSSL 076	Palmitic	2017	-21.08
CSSL 011	Palmitic	2017	-25.153	CSSL 079	Arachidic	2016	22.723
CSSL 013	Palmitic	2016	-17.169	CSSL 079	Palmitic	2016	-13.983
CSSL 013	Palmitic	2017	-22.773	CSSL 079	Arachidic	2017	47.997
CSSL 014	Arachidic	2016	26.24	CSSL 079	Palmitic	2017	-16.775
CSSL 014	Palmitic	2016	-12.087	CSSL 084	Eicosenoic	2016	48.567
CSSL 014	Arachidic	2017	50.286	CSSL 084	Iodine	2016	-7.971
CSSL 014	Palmitic	2017	-15.708	CSSL 084	Oleic	2016	35.587
CSSL 015	Palmitic	2016	-15.121	CSSL 084	Linoleic	2016	-37.552
CSSL 015	Palmitic	2017	-14.243	CSSL 084	O/L	2016	117.367
CSSL 016	Palmitic	2016	-10.679	CSSL 084	Palmitic	2016	-27.099
CSSL 016	Palmitic	2017	-17.358	CSSL 084	O/L	2017	118.487
CSSL 020	Palmitic	2016	-18.298	CSSL 084	Eicosenoic	2017	51.017
CSSL 020	Palmitic	2017	-16.714	CSSL 084	Iodine	2017	-9.388
CSSL 021	Palmitic	2016	-23.484	CSSL 084	Linoleic	2017	-38.244
CSSL 021	Palmitic	2017	-23.042	CSSL 084	Oleic	2017	34.606
CSSL 022	Palmitic	2016	-20.455	CSSL 084	Palmitic	2017	-33.833
CSSL 022	Palmitic	2017	-18.742	CSSL 085	Arachidic	2016	26.06
CSSL 023	Palmitic	2016	-14.506	CSSL 085	Palmitic	2016	-15.138
CSSL 023	Palmitic	2017	-17.093	CSSL 085	Arachidic	2017	54.456
CSSL 024	Palmitic	2016	-21.033	CSSL 085	Palmitic	2017	-23.699
CSSL 024	Palmitic	2017	-24.043	CSSL 086	Palmitic	2016	-16.474
CSSL 025	Palmitic	2016	-16.953	CSSL 086	Palmitic	2017	-23.038
CSSL 025	Palmitic	2017	-16.996	CSSL 090	Palmitic	2016	-21.34
CSSL 027	Palmitic	2016	-14.867	CSSL 090	Palmitic	2017	-24.025
CSSL 027	Palmitic	2017	-16.775	CSSL 091	Arachidic	2016	26.42
CSSL 028	Palmitic	2016	-16.501	CSSL 091	Palmitic	2016	-23.019
CSSL 028	Palmitic	2017	-17.014	CSSL 091	Arachidic	2017	75.225
CSSL 031	Behenic	2016	22.105	CSSL 091	Palmitic	2017	-25.49
CSSL 031	Arachidic	2016	28.314	CSSL 095	Arachidic	2016	22.633
CSSL 031	Palmitic	2016	-13.342	CSSL 095	Palmitic	2016	-15.869
CSSL 031	Behenic	2017	37.007	CSSL 095	Arachidic	2017	53.23
CSSL 031	Palmitic	2017	-17.04	CSSL 095	Palmitic	2017	-26.574
CSSL 031	Arachidic	2017	69.011	CSSL 096	Palmitic	2016	-14.863
CSSL 032	Palmitic	2016	-22.423	CSSL 096	Palmitic	2017	-16.14
CSSL 032	Palmitic	2017	-24.458	CSSL 098	Palmitic	2016	-16.014
CSSL 033	Palmitic	2016	-16.024	CSSL 098	Palmitic	2017	-17.666
CSSL 033	Palmitic	2017	-19.871	CSSL 100	Iodine	2016	-6.366
CSSL 034	Stearic	2016	46.338	CSSL 100	Eicosenoic	2016	41.653
CSSL 034	Arachidic	2016	29.838	CSSL 100	Linoleic	2016	-30.044
CSSL 034	Palmitic	2016	-21.864	CSSL 100	Oleic	2016	28.507
CSSL 034	Stearic	2017	83.813	CSSL 100	O/L	2016	83.36
CSSL 034	Arachidic	2017	64.513	CSSL 100	Palmitic	2016	-24.418
CSSL 034	Palmitic	2017	-22.667	CSSL 100	Iodine	2017	-6.889
CSSL 036	Palmitic	2016	-11.78	CSSL 100	O/L	2017	126.05
CSSL 036	Palmitic	2017	-19.254	CSSL 100	Eicosenoic	2017	40.552

Table 2. Continued.

Sample	Trait	Year	Effect (%)	Sample	Trait	Year	Effect (%)
CSSL 037	Arachidic	2016	33.814	CSSL 100	Linoleic	2017	-34.297
CSSL 037	Palmitic	2016	-20.238	CSSL 100	Oleic	2017	34.903
CSSL 037	Arachidic	2017	49.877	CSSL 100	Palmitic	2017	-30.87
CSSL 037	Palmitic	2017	-18.989	CSSL 101	Palmitic	2016	-20.554
CSSL 039	Stearic	2016	63.623	CSSL 101	Palmitic	2017	-19.818
CSSL 039	Arachidic	2016	34.152	CSSL 102	Palmitic	2016	-13.163
CSSL 039	Palmitic	2016	-17.626	CSSL 102	Palmitic	2017	-16.802
CSSL 039	Stearic	2017	86.145	CSSL 104	Palmitic	2016	-14.903
CSSL 039	Arachidic	2017	60.098	CSSL 104	Palmitic	2017	-14.156
CSSL 039	Palmitic	2017	-23.743	CSSL 109	Palmitic	2016	-19.183
CSSL 040	Stearic	2016	99.864	CSSL 109	Palmitic	2017	-21.027
CSSL 040	Palmitic	2016	-23.981	CSSL 110	Palmitic	2016	-13.423
CSSL 040	Arachidic	2016	58.405	CSSL 110	Palmitic	2017	-16.74
CSSL 040	Arachidic	2017	68.118	CSSL 111	Iodine	2016	-4.413
CSSL 040	Stearic	2017	124.355	CSSL 111	Eicosenoic	2016	36.425
CSSL 040	Palmitic	2017	-29.234	CSSL 111	Linoleic	2016	-25.576
CSSL 044	Palmitic	2016	-14.75	CSSL 111	O/L	2016	71.567
CSSL 044	Palmitic	2017	-19.166	CSSL 111	Oleic	2016	26.746
CSSL 047	Palmitic	2016	-11.094	CSSL 111	Palmitic	2016	-27.848
CSSL 047	Palmitic	2017	-17.402	CSSL 111	Eicosenoic	2017	34.157
CSSL 051	Palmitic	2016	-21.421	CSSL 111	Iodine	2017	-7.077
CSSL 051	Palmitic	2017	-20.621	CSSL 111	O/L	2017	123.277
CSSL 052	Palmitic	2016	-14.173	CSSL 111	Linoleic	2017	-35.882
CSSL 052	Palmitic	2017	-17.18	CSSL 111	Oleic	2017	36.838
CSSL 056	Arachidic	2016	30.298	CSSL 111	Palmitic	2017	-32.995
CSSL 056	Palmitic	2016	-17.458	CSSL 112	Palmitic	2016	-19.169
CSSL 056	Arachidic	2017	73.671	CSSL 112	Palmitic	2017	-20.736
CSSL 056	Palmitic	2017	-26.557	CSSL 113	Palmitic	2016	-19.724
CSSL 058	Arachidic	2016	24.436	CSSL 113	Palmitic	2017	-26.639
CSSL 058	Palmitic	2016	-17.639	CSSL 114	Palmitic	2016	-20.69
CSSL 058	Arachidic	2017	61.733	CSSL 114	Palmitic	2017	-19.059
CSSL 058	Palmitic	2017	-22.403	CSSL 115	Stearic	2016	47.433
CSSL 059	Arachidic	2016	25.699	CSSL 115	Arachidic	2016	37.151
CSSL 059	Palmitic	2016	-16.718	CSSL 115	Palmitic	2016	-21.078
CSSL 059	Arachidic	2017	71.3	CSSL 115	Stearic	2017	74.246
CSSL 059	Palmitic	2017	-26.821	CSSL 115	Arachidic	2017	50.123
CSSL 060	Arachidic	2016	28.133	CSSL 115	Palmitic	2017	-17.155
CSSL 060	Palmitic	2016	-19.182	CSSL 116	Palmitic	2016	-12.403
CSSL 060	Arachidic	2017	79.967	CSSL 116	Palmitic	2017	-16.149
CSSL 060	Palmitic	2017	-23.717	CSSL 118	Palmitic	2016	-18.803
CSSL 061	Palmitic	2016	-15.653	CSSL 118	Palmitic	2017	-16.528
CSSL 061	Palmitic	2017	-19.677	CSSL 119	Palmitic	2016	-19.525
CSSL 063	Palmitic	2016	-18.154	CSSL 119	Palmitic	2017	-15.073
CSSL 063	Palmitic	2017	-24.731	CSSL 120	Palmitic	2016	-17.268
CSSL 066	Stearic	2016	51.63	CSSL 120	Palmitic	2017	-20.312
CSSL 066	Arachidic	2016	29.306	CSSL 121	Palmitic	2016	-15.68
CSSL 066	Palmitic	2016	-13.306	CSSL 121	Palmitic	2017	-18.557

For traits with significant differences ($P \leq 0.05$) in the population in both 2016 and 2017, individual lines were compared with Fleur 11 to determine the effect of introgressions, which were in turn estimated by calculating the percent increase or decrease relative to Fleur 11. Shown are 210 line by trait by year differences that were apparent.

fatty acids in defining the O/L profile (Isleib *et al.*, 2006). The O/L of Fleur 11 which is the genetic background scaffolding the CSSLs was 1.3. This puts it in the lower spectrum of the normal range of 1.0-2.5 for most commercial cultivated varieties and particularly the spanish types (López *et al.*, 2000; Davis *et al.*, 2016). The mean of the CSSLs was 1.5, indicating that a majority of the lines were

Table 3. Values of oleic/linoleic and associated fatty acids for the three top lines, CSSL 84, CSSL 100 and CSSL 111.

Sample	Year	Oleic (%)	Linoleic (%)	O/L	Mean O/L	Palmitic (%)	Stearic (%)	Arachidic (%)	Behenic (%)	Eicosenoic (%)	Iodine
CSSL 084	2016	62.11	20.91	2.99	2.94	8.97	2.24	1.1	2.06	0.98	89.65
CSSL 084	2017	59.98	20.83	2.89		8.34	3.75	1.53	2.49	1.15	87.66
CSSL 100	2017	60.11	22.16	2.99	2.76	8.71	2.8	1.25	2.33	1.07	90.08
CSSL 100	2016	58.87	23.43	2.52		9.3	2.43	1.19	2.3	0.93	91.21
CSSL 111	2017	60.97	21.63	2.95	2.66	8.44	3.19	1.3	2.07	1.03	89.9
CSSL 111	2016	58.06	24.93	2.36		8.88	2.37	1.16	2.15	0.9	93.11
Fleur 11[a]	2016	45.81	33.49	1.38	1.35	12.31	2.73	1.23	2.43	0.66	97.41
Fleur 11[a]	2017	44.56	33.73	1.32		12.6	3.24	1.36	2.59	0.76	96.75
NM Valencia[b]	2017	43.00	35.52	1.21	1.21	10.59	3.96	1.62	2.93	0.88	98.51
OLin[c]	2017	71.11	10.10	7.04	7.04	7.34	4.06	1.69	2.82	1.21	78.66

[a]Fleur 11 is the cultivated genetic background of the CSSL population
[b]NM Valencia is a low O/L check variety
[c]OLin is a high O/L check variety

normal and similar to Fleur 11 (Table 1). However, three lines, CSSL 84, CSSL 100 and CSSL 111 had ratios of 2.94, 2.76 and 2.66 respectively (Table 3). These values are higher than for the low O/L check New Mexico Valencia A which had a score of 1.21. They are also lower than the score of the high O/L check OLin which had a value of 7.04. Though within the normal range, the values of these higher O/L CSSLs translate to more than 100% increase relative to Fleur 11 which is the standard of comparison (Table 2). The values were also comparable to higher ranked normal O/L runner varieties (Branch *et al.*, 1990; Andersen *et al.*, 1998).

As noted above, these fatty acid profiles are under genetic control, therefore it follows that introgressions present therein have introduced variations that have increased the O/L. The high O/L trait is the result of two recessive genes ol_1 and ol_2 also referred to as *ahFAD2A* and *ahFAD2B*, respectively (Moore and Knauft, 1989; Jung *et al.*, 2000a; Chu *et al.*, 2009). These genes encode for Δ^{12}-desaturase enzyme that catalyzes the initial step in polyunsaturated fatty acid biosynthesis, which is conversion of oleate to linoleate. In US high O/L cultivars, the mutation in *ahFAD2A* involves a single base G to A substitution at position 448 (Chu *et al.*, 2007). For the *ahFAD2B,* either of two mutations achieve desaturase suppression. The first is a position 441_442 A insertion while the second is a MITE insertion at position 665 (Chu *et al.*, 2009). These mutations result in increase of oleic acid above the normal range of 36-67% and reduction of linoleic acid below the normal range of 15-43% (Norden *et al.*, 1987; Moore and Knauft, 1989; Ray *et al.*, 1993). It is likely that introgressions in these three lines may not be associated with the canonical mutations on *ahFAD2A* and *ahFAD2B* (Pandey *et al.*, 2014; Shasidhar *et al.*, 2017). Genotyping work on the lines (Gimode *et al.*, unpubl. data) indicates that CSSL 84 has multiple introgressions on nearly all chromosomes, making it difficult to decipher specific polymorphisms that contribute to the trait. CSSL 100 has clear introgressions on the upper and lower arms of chromosomes A10 and B10. There is co-occurrence of polymorphism in these chromosomes between CSSL 100 and CSSL 84. This makes these regions good candidates for further study of the genetic basis for the trait. Unfortunately, clear introgression patterns are yet to be observed in CSSL 111. Presence of multiple introgressions in these lines indicates the likelihood of multiple genes interacting with the genetic background of Fleur 11 to influence variation in the O/L trait as similarly observed by Isleib *et al.*, (2006). The results of this study suggest the potential involvement of new genetic polymorphisms associated with the O/L trait. While these polymorphisms do not radically increase the O/L, understanding their basis may facilitate potentiation of the conventional high O/L trait.

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