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RESEARCH ARTICLE

Fatty acid desaturase-2 (*ahFAD2*) mutant alleles in peanut (*Arachis hypogaea* L.) pre-breeding lines: an insight into the source, features, discourse, and selection of novel pre-breeding lines

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Abstract High oleic peanuts and derived food products offer longer shelf life benefits to the food processing industry in addition to multiple health benefits to the consumers. The two mutant alleles, *ahFAD2A* and *ahFAD2B* control composition of oleic, linoleic and palmitic acid content in peanut. A total of 563 peanut pre-breeding lines were tested for the presence *ahFAD2A* and *ahFAD2B* mutant alleles using allele specific markers. The *ahFAD2A* mutant allele was present in 82 lines, while none of these lines had *ahFAD2B* mutant allele. Among botanical types, *ahFAD2A* mutant allele was more frequent in lines with Virginia growth habit than Spanish bunch although no correlation of *ahFAD2A* mutant allele with high oleic acid content and growth habit could be established. Oleic and linoleic acid content in 82 pre-breeding lines ranged from 39.70 to 62.70% and 17.76 to 31.95%, respectively, with maximum oleic to linoleic acid ratio of 4. Oleic acid was found to be negatively correlated with linoleic and palmitic acid. Further, pre-breeding lines with *ahFAD2A* mutant allele, high oleic content and high oleic to linoleic ratio were investigated and novel lines were identified for resistance to late leaf spot, short duration, higher pod yield and other yield related traits. These novel pre-breeding lines can be used as a potential donor in peanut improvement programme and to diversify the primary gene pool including initiating further research on induction of fresh *ahFAD2B* mutant allele.

Keywords Peanut · Oleic acid · Linoleic acid · Palmitic acid · Oil quality · Shelf life · Pre-breeding lines

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Introduction

Peanut (*Arachis hypogaea* L.) plays a pivotal role as both an oilseed and food crop worldwide. It offers livelihood to millions, especially in the semi-arid tropic (SAT) regions of the world. About 47.09 million tonnes of peanut are produced from 27.94 million hectares worldwide (FAOSTAT 2017); China, India, Nigeria, and the United States of America (USA) together account for ~ 70% of the global peanut production. The fatty acid composition of the oil extracted from peanut seed determines the quality and shelf-life of the oil and peanut-based food products. Oleic acid, a mono-unsaturated fatty acid (MUFA), and linoleic acid, a polyunsaturated fatty acid (PUFA), constitute ~ 80% of the peanut fat composition (Mukri et al. 2012; Bera et al. 2018a). Besides, palmitic acid, a saturated fatty acid (SFA), contributes ~ 10% of the total fat in peanut. Thus, palmitic, oleic and linoleic acids together constitute ~ 90% of the total fats in peanuts. Primarily, a high oleic acid/linoleic acid (O/L) ratio results in the longer shelf-life of the oil, defining its quality. Also, oil with high O/L ratio is virtually favored for consumption due to its multiple health benefits, since higher SFAs increases serum low-density lipoproteins (LDL) cholesterol (known as bad cholesterol) level in the blood (Johnson and Saikia 2008; Bera et al. 2018a). Moreover, a higher proportion of linoleic acid is prone to oxidative rancidity resulting in thermodynamically unstable compound upon boiling (Kratz et al. 2002; Bera et al. 2018a). Furthermore, a flux in linoleic acid results in the formation of trans-fatty acids, which could promote cardiovascular diseases (CVD). On the contrary, consumption of high oleic peanut oil or derived-food products reduces the risk of heart diseases, tumor genesis and inflammatory diseases (Yamaki et al. 2005). In addition, the oxidative stability of peanut oil and derived-food products is directly correlated with a high O/L ratio (O'Keefe et al. 1993). Thus, food products with a high oleic and O/L ratio will have longer shelf-life and greater health benefits than normal peanut (Bolton and Sanders 2002; Mozingo et al. 2004). Moreover, peanut oil with a lower concentration of palmitic acid, a saturated fat, lowers the risk of CVD (Bera et al. 2018a).

In peanuts, the Δ-12-desaturase enzyme encoded by *ahFAD2A* and *ahFAD2B* alleles, located on A-genome (linkage group a09) and B-genome (linkage group b09) of peanut, respectively, catalyzes oleic to linoleic acid conversion (Jung et al. 2000a; Lopez et al. 2000; Pandey et al. 2014). The inactivation of these alleles is required for high oleic trait expression in peanut as mutated *ahFAD2A* and *ahFAD2B* alleles fail to produce Δ-12-desaturase enzyme (Jung et al. 2000a, b; Lopez et al. 2000; Chu et al. 2009) and subsequently, conversion of oleic to linoleic acid ceases. Thus in peanuts, the presence of these mutant alleles in a genotype enhances oleic acid and reduces linoleic acid (Gorbet and Knauft 1997; Wang et al. 2013, 2015). It is also reported that the increase in oleic acid further reduces palmitic acid (Janila et al. 2016; Bera et al. 2018a). Since oleic, linoleic, and palmitic acids comprise 90% of the total fat in peanut, reduction in both linoleic and palmitic acids would elevate oleic acid content.

Breeding peanut for increased oleic acid and reduced linoleic and palmitic acid content are essential for healthier peanuts. Norden et al. (1987) first reported a peanut mutant, F435 containing 80% oleic acid and 2% linoleic acid, which later helped in developing SunOleic 95R in the USA (Gorbet and Knauft 1997). Over the years, high oleic peanuts have been developed using molecular breeding (Chu et al. 2011; Mienie and Pretorius 2013; Janila et al. 2016; Xiuzhen et al. 2016; Yang et al. 2017; Bera et al. 2018a, 2019; Nawade et al. 2019; Shasidhar et al. 2020). Recently, two high oleic varieties, namely, Girnar 4 and Girnar 5, have been identified for commercial cultivation in India (Proceedings, annual group meeting of all India coordinated research program on groundnut, 25–27 May 2019, Andhra University, Visakhapatnam, India).

So far, high oleic peanut developed worldwide have used single source of *ahFAD2B* mutant allele originating from F435 since there is no reports for its alternative source. Associated molecular markers have been used widely to search for an alternative source of *ahFAD2B* mutant allele in peanut but remained unsuccessful. However, several alternate sources for *ahFAD2A* mutant allele have been reported (Chu et al. 2007; Mukri et al. 2012; Nawade et al. 2016). Looking at this, identification of elite pre-breeding lines (PBLs) containing *ahFAD2* mutant alleles and elevated oleic acid content seems essential for boosting the peanut breeding program. Therefore, an attempt was made to characterize PBLs developed by inter-specific/intra-specific hybridization and mutations for *ahFAD2A* and

ahFAD2B mutant alleles, high oleic acid content, high oleic to linoleic acid ratio, and high oil content along with resistance to late leaf spot (LLS) disease, pod yield, and other yield-related traits, to finally identify novel PBLs.

Materials and methods

Plant materials

A total of 563 PBLs available at ICAR-Directorate of Groundnut Research (ICAR-DGR), Junagadh, along with SunOleic 95R-a high oleic ($\sim$ 80%) genotype were used in this study. Out of 563 PBLs, 526 were developed by inter-specific/intra-specific hybridization while 37 were developed by mutagenesis using EMS (ethyl methane sulfonate). We developed PBLs of interspecific/intra-specific origin under several breeding objectives over 35 years at ICAR-DGR, Junagadh. We hybridized several wild species with cultivated peanut, which resulted in large number of desirable inter-specific PBLs. In general, wild *Arachis* species are spreading and perennial type. Hybridization between wild *Arachis* species with cultivated peanut produced segregants of different growth habit namely Spanish bunch (SB), Virginia bunch (VB) and Virginia runner (VR) depending on female parent used. Similarly, we effected different intra-specific crosses to pyramid desirable alleles for various biotic and abiotic stresses in peanut and developed several desirable intra-specific PBLs over 35 years at ICAR-DGR, Junagadh. These intra-specific crosses also produced segregants/PBLs of SB, VB and VR growth habit depending on parent used. Moreover, we also used mutation breeding to induce fresh alleles/traits for different biotic and abiotic stresses in peanut over 35 year at ICAR-DGR, Junagadh and developed several desirable PBLs of SB, VB and VR growth habit. These inter-specific/intra-specific/mutant PBLs are being regularly used as donors in our different ongoing peanut improvement program (Bera et al. 2008, 2010, 2011, 2012, 2014, 2016). However, in the course of developing these 563 PBLs our breeding objectives never directed to *ahFAD2* alleles since it was reported later (Chen et al. 2010). Hence, we were ignorant about the presence of *ahFAD2* alleles in these PBLs, which prompted us to characterize the PBLs for presence of *ahFAD2* alleles. Among 526 inter-specific/intra-specific PBLs characterized under this study 172, 349, and 5 were of SB, VB, and VR growth habit, respectively. Among the 37 mutant PBLs, 6 and 31 were of SB and VB growth habit, respectively. Altogether, we screened 178 SB, 380 VB, and 5 VR PBLs (in total 563) initially for presence of *ahFAD2A* and *ahFAD2B* mutant alleles. Later, PBLs positive for either or both *ahFAD2* mutant alleles were further characterized for the fatty acid profile, oil content, disease resistance, pod yield, and other yield-related traits.

DNA extraction and marker genotyping

All the 563 PBLs were planted during the *Kharif* season in 2016 at ICAR-DGR, Junagadh. Fresh leaf samples from 10 to 15 days old plants were collected for DNA extraction. The modified Cetyltrimethyl Ammonium Bromide (CTAB) method was used for DNA extraction (Mace et al. 2003). The quality of DNA was checked on 0.8% agarose gel (Lonza, USA), and its concentration was estimated on an ND100 Spectrophotometer (NanoDrop Technology, USA). The working concentration was further adjusted to 20 ng μL^{-1}.

Genotyping using AS-PCR markers

PBLs and SunOleic 95R were genotyped using allele specific-polymerase chain reaction (AS-PCR) markers (Chen et al. 2010) (Table 1). The polymerase chain reaction (PCR) was carried out in C1000 Thermal cycler (BIO-RAD, USA) with a reaction volume of 25 µL (Bera et al. 2018a). The amplified DNA fragments along with 100 bp DNA marker (Thermo Scientific, USA) were separated on a 2% horizontal agarose gel (Lonza, USA). Gel electrophoresis was carried out in 1X TBE buffer at 100 V current for 1–2 h. Ethidium bromide (EtBr) was used for staining the DNA fragments, and the gel was scanned using a laser scanner (Fujifilm FLA 5100, Japan) for scoring.

Oil and fatty acid analysis

Sound matured kernels of selected 82 PBLs were analyzed for oil content and fatty acid profile using gas chromatography (GC 700, Thermo Fisher, USA) with

Table 1 Allele-specific polymorphic chain reaction markers used for selecting plants with *ahFAD2A* and *ahFAD2B* mutant alleles

Allele	Markers	Sequence (5′ to 3′)	Wild allele	Size of mutant allele	Reference
ahFAD2A	F435-F	ATCCAAGGCTGCATTCTCAC	Null allele	203 bp	Chen et al. (2010)
	F435SUB-R	TGGGACAAACACTTCGTT			
ahFAD2B	F435-F	ATCCAAGGCTGCATTCTCAC	Null allele	195 bp	
	F435INS-R	AACACTTCGTCGCGGTCT			

a flame ionization detector (FID) as described by Bera et al. (2018a).

Screening for resistance to late leaf spot disease and evaluation of agronomic traits

The selected 82 PBLs along with check genotypes were replanted during the *Kharif* season in 2017 and 2018. In both the years, the experiment was planted in RBD with three replications. Each genotype was planted in a single line, 5-meter in length. Susceptible check (cultivar GG-2) for LLS disease was planted after every ten lines of test genotypes to increase the disease pressure. Besides, LLS inoculum mixed in water was sprayed 35 days after sowing (Khedikar et al. 2010), and the LLS disease score was recorded on a 1–9 scale 90 days after sowing (DAS) (Subbarao et al. 1990).

Moreover, recommended check genotypes for additional traits of interest were planted once in each replication. The days to 50% flowering (DFF) was recorded visually by visiting the field daily, starting from the initiation of flowering till attaining 50% flowering. DFF was calculated by counting the number of days from sowing to the attainment of 50% flowering. Data on pod weight and hundred kernel weight (HKW) were recorded at harvest, and the plant population of each PBL was recorded during harvesting. Pod weight of each PBL was recorded after proper drying of the pods ($\sim$ 8% pod moisture) in the sun in terms of pod weight per 10 plants since each PBL was planted in a single row, and the plant population was inconsistent. Pod weight per 10 plants was calculated by dividing pod weight by the line with plant population and multiplying by 10. HKW was measured by weighing 100 sound matured kernels of each line, and testa color was recorded visually and grouped as rose, red, and variegated.

Statistical analysis

Variance and simple correlation coefficients were analyzed using PAST software, ver. 3.25 (Hammer et al. 2001). Correlation analysis among different fatty acids was performed using the 'psych' package (Revelle 2019) in R (R core team 2018).

Results

AS-PCR assay

A total of 563 PBLs along with SunOleic 95R were screened using diagnostic AS-PCR markers associated with *ahFAD2A* and *ahFAD2B* mutant alleles. AS-PCR assay revealed the presence of *ahFAD2A* mutant allele in 80 out of 526 PBLs of inter-specific/inter-generic origins and in two PBLs out of 37 mutants. Altogether, 82 PBLs were found positive for *ahFAD2A* mutant allele (Table 2) and no PBL was positive for *ahFAD2B* mutant allele. Furthermore, among botanical groups, 6.2% SB, 18.4% VB, and 20% VR were found positive for *ahFAD2A* mutant allele. Finally, the 82 PBLs positive for *ahFAD2A* mutant allele were selected for further analysis.

Biochemical analysis for oil content and fatty acids

Oil content and fatty acid profile of any peanut genotype are of immense importance, and the improvement of these traits could be a source of high income to all the peanut stakeholders. Oil content and fatty acid profile were analyzed in selected 82 PBLs during *Kharif* seasons in 2017 and 2018. The oil content varied from 40 to 57% in 2017 and 49 to 55% in 2018. Very high (> 52%) oil content was reported

Table 2 Growth habit and pedigree of the 82 selected pre-breeding lines

S. no.	Name	Growth habit	Pedigree
A: Interspecific/intervarietal lines			
1.	NRCGCS-77	SB	CT7-1 × (SBXI × *A. kretschmeri*)
2.	NRCGCS-78	SB	CT7-1 × SBXI
3.	NRCGCS-79	SB	CT7-1 × SBXI
4.	NRCGCS-85	SB	CT7-1 × SBXI × *A. diogoi*
5.	NRCGCS-156	SB	GAUG 10 × (PBDR 25 × *A. cardinansii*)
6.	NRCGCS-446	SB	J 11 × *A. pussila*
7.	NRCGCS-186	SB	C364 × (PBDR 26 × *A. oteroi*)
8.	NRCGCS-188	SB	C364 × PBDR 27
9.	NRCGCS-308	SB	J 11 × *A. kretschmeri*
10.	NRCGCS-166	SB	GG 2 × *A. chacoensis*
11.	NRCGCS-03	VB	CT7-1 × SBXI
12.	NRCGCS-20	VB	CT7-1 × SBXI
13.	NRCGCS-30	VB	CT7-1 × SBXI
14.	NRCGCS-32	VB	CT7-1 × SBXI
15.	NRCGCS-37	VB	CT7-1 × SBXI
16.	NRCGCS-38	VB	CT7-1 × SBXI
17.	NRCGCS-54	VB	CT7-1 × (SBXI × *A. pusilla*)
18.	NRCGCS-61	VB	CT7-1 × SBXI
19.	NRCGCS-68	VB	CT7-1 × SBXI
20.	NRCGCS-70	VB	CT7-1 × SBXI
21.	NRCGCS-72	VB	CT7-1 × SBXI
22.	NRCGCS-73	VB	CT7-1 × (SBXI × *A. correntina*)
23.	NRCGCS-105	VB	CT7-1 × SBXI
24.	NRCGCS-107	VB	CT7-1 × SBXI
25.	NRCGCS-136	VB	GG 2 × *A. chacoensis*
26.	NRCGCS-137	VB	J 11 × *A. chacoensis*
27.	NRCGCS-139	VB	C 364 × NcAc 17090
28.	NRCGCS-144	VB	C 364 × NcAc 17090
29.	NRCGCS-149	VB	C 364 × PBDR 25
30.	NRCGCS-152	VB	J 11 × *A. villosa*
31.	NRCGCS-154	VB	BAU 12 × Chico
32.	NRCGCS-155	VB	BAU 12 × Chico
33.	NRCGCS-160	VB	GAUG 10 × CGC 4018
34.	NRCGCS-161	VB	GG 2 × *A. manfredi*
35.	NRCGCS-182	VB	J 11 × (J 11 × *A. cardinansii*)
36.	NRCGCS-183	VB	J 11 × (J 11 × *A. cardinansii*)
37.	NRCGCS-196	VB	GAUG 10 × (CGC 4018 × *A. correntina*)
38.	NRCGCS-199	VB	J 11 × *A. duranensis*
39.	NRCGCS-200	VB	BAU-12 × Chico
40.	NRCGCS-218	VB	GG 2 × *A. chacoensis*
41.	NRCGCS-219	VB	C364 × NcAc 17090

Table 2 continued

S. no.	Name	Growth habit	Pedigree
42.	NRCGCS-220	VB	GG 2 × *A. duranensis*
43.	NRCGCS-234	VB	GAUG 10 × PBDR 26
44.	NRCGCS-239	VB	GAUG 10 × PBDR 27
45.	NRCGCS-245	VB	GG 2 × *A. cardinasii*
46.	NRCGCS-246	VB	GG 2 × *A. cardinasii*
47.	NRCGCS-252	VB	CS-21
48.	NRCGCS-256	VB	KPI - TGK
49.	NRCGCS-257	VB	Code-26
50.	NRCGCS-260	VB	J 11 × *A. cardinasii*
51.	NRCGCS-264	VB	Deep red mutant × Purple variegated mutant
52.	NRCGCS-265	VB	Deep red mutant × Purple variegated mutant
53.	NRCGCS-266	VB	Deep red mutant × Purple variegated mutant
54.	NRCGCS-269	VB	Deep red mutant × Purple variegated mutant
55.	NRCGCS-270	VB	Deep red mutant × Purple variegated mutant
56.	NRCGCS-280	VB	Deep red mutant × Purple variegated mutant
57.	NRCGCS-281	VB	Deep red mutant × (Purple variegated mutant × *A. duranensis*)
58.	NRCGCS-282	VB	Deep red mutant × Purple variegated mutant
59.	NRCGCS-285	VB	Purple tan mutant × Deep red mutant
60.	NRCGCS-303	VB	J 11 × *A. kretschmeri*
61.	NRCGCS-311	VB	J 11 × *A. kretschmeri*
62.	NRCGCS-315	VB	J 11 × *A. duranensis*
63.	NRCGCS-319	VB	J 11 × *A. duranensis*
64.	NRCGCS-327	VB	J 11 × *A. duranensis*
65.	NRCGCS-349	VB	Packered leaf mutant × Crincle leaf mutant
66.	NRCGCS-409	VB	J 11 × *A. duranensis*
67.	NRCGCS-411	VB	J 11 × *A. duranensis*
68.	NRCGCS-412	VB	J 11 × *A. duranensis*
69.	NRCGCS-414	VB	J 11 × *A. duranensis*
70.	NRCGCS-417	VB	J 11 × *A. duranensis*
71.	NRCGCS-424	VB	GG 20 × CS-19
72.	NRCGCS-426	VB	J 11 × *A. duranensis*
73.	NRCGCS-432	VB	J 11 × *A. duranensis*
74.	NRCGCS-434	VB	J 11 × *A. duranensis*
75.	NRCGCS-461	VB	J 11 × *A. pusilla*
76.	NRCGCS-475	VB	J 11 × *A. pusilla*
77.	NRCGCS-492	VB	J 11 × *A. pusilla*
78.	NRCGCS-532	VB	J 11 × *A. pusilla*
79.	NRCGCS-536	VB	J 11 × *A. pusilla*
80.	NRCGCS-430	VR	J 11 × *A. duranensis*
B: Mutant lines			
81.	NRCGCS-404	SB	GG 2 mutant
82.	NRCGCS-385	VB	GG 2 mutant

VB Virginia bunch, *SB* Spanish bunch

in 12 PBLs namely, NRCGCS-85, NRCGCS-182, NRCGCS-234, NRCGCS-256, NRCGCS-270, NRCGCS-308, NRCGCS-414, NRCGCS-424, NRCGCS-426, NRCGCS-446, NRCGCS-461, and NRCGCS-475 (Table 3). However, the highest oil content (56%) was observed in NRCGCS-414, an inter-specific VB breeding line. Oleic acid content varied from 41 to 63% in 2017, and 39 to 65% in 2018. More than 60% of oleic acid content was observed in eight PBLs, namely, NRCGCS-385, NRCGCS-409, NRCGCS-411, NRCGCS-412, NRCGCS-414, NRCGCS-424, NRCGCS-461, and NRCGCS-492 (Table 4). On the other hand, linoleic acid content varied from 17 to 36% in 2017 and 16 to 37% in 2018. Less than 20% linoleic acid was observed in six PBLs, namely, NRCGCS-411, NRCGCS-412, NRCGCS-414, NRCGCS-424, NRCGCS-461, and NRCGCS-492 (Table 4). Likewise, palmitic acid varied from 9 to 14% in 2017 and 8 to 12% in 2018. Less than 10% of palmitic acid was observed in 11 PBLs. Oleic to linoleic acid ratio varied from 1 to 4. The maximum average O/L ratio (3.5) was observed in NRCGCS-411 and NRCGCS-461. Thus, NRCGCS-411 and NRCGCS-461 had higher oleic acid content, higher O/L ratio, and lower palmitic acid content (Table 4). NRCGCS-411 is an interspecific derivative of *A. hypogaea* × *A. duranensis*, while NRCGCS-461 is an interspecific derivative of *A. hypogaea* × *A. pusilla* (Table 2).

Correlations analysis between fatty acids

Earlier studies on correlation coefficients and the magnitude and direction of the genetic correlations (positive or negative) between traits help in selective decisions and subsequently planning breeding strategies. Oleic acid had negative correlation with the majority of the SFAs, including palmitic acid (r = − 0.867). A significant negative correlation was observed between oleic acid and linoleic acid (r = − 0.991), oleic and gadoleic acids (r = − 0.213), and oleic and palmitoleic acids (r = − 0.248). On the contrary, a significant positive correlation was observed between palmitic acid and linoleic acid (r = 0.852); stearic acid and arachidic acid (r = 0.773); arachidic acid and behenic acid (r = 0.509); erucic acid and gadoleic acid (r = 0.844); lignoceric acid and gadoleic acid (r = 0.863); lignoceric acid and behenic acid (r = 0.528) and lignoceric acid and erucic acid (r = 0.748). A significant negative correlation was also observed between stearic acid and gadoleic acid (r = − 0.623), as well as stearic acid and erucic acid (r = − 0.567) (Fig. 1).

Yield and yield-related traits

High yield remains one of the key economic traits in peanut that defines high oil yield and maximum profitability to the farmers. High pod yield (≥ 100 g/10 plants) was observed in six genotypes, namely, NRCGCS-37, NRCGCS-281, NRCGCS-285, NRCGCS-385, NRCGCS-409, and NRCGCS-412 (Table 5). In most groundnut growing states in India, the high yield was achieved through a progressive improvement in pod or kernel weight. In 2017, the pod weight of PBLs varied from 14 to 118 g with an average of 68.5 g, while in 2018, it ranged from 20 to 114 g with an average of 69.9 g.

Hundred kernel weight (HKW) is a significant yield contributing trait in peanuts, since higher kernel weight is preferred for table purpose and internationally, hand-picked selection (HPS) peanuts are in great demand. Wide variation was observed in HKW in both the seasons. In 2017, HKW varied from 16 to 84 g, with an average of 40.5 g. A similar trend was observed in 2018 with variation from 23 to 82 g and an average of 40.9 g (Table 5). Maximum HKW was observed in NRCGCS-281, which is a novel peanut germplasm registered for its large kernel size (Fig. 2) (Bera et al. 2018c).

In peanuts, earliness is associated with DFF. In India, peanut varieties with shorter duration (90−100 days) are suitable for *rabi* or summer (post rainy) cultivation. No significant variation was observed in DFF between seasons. However, among PBLs, DFF varied from 16 to 27 days. DFF less than 20 days (on par with short duration check) was observed in three PBLs, namely, NRCGCS-199, NRCGCS-260 and NRCGCS-404 (Table 6).

The color of the seed coat/testa is another essential diagnostic character and market trait. In general, peanuts with rose color testa is preferred for oil extraction since darkening reduces its value and market acceptability. The majority of the PBLs had

Table 3 Oil content of the selected 82 pre-breeding lines during *kharif*, 2017 and *kharif*, 2018

S. no.	Genotype	Oil %		
		Kharif, 2017	*Kharif*, 2018	Mean
1.	NRCGCS-77	50.5	51.1	50.80
2.	NRCGCS-78	50.0	51.0	50.50
3.	NRCGCS-79	50.0	51.3	50.65
4.	NRCGCS-85	52.0	52.7	52.35
5.	NRCGCS-156	48.0	50.2	49.10
6.	NRCGCS-404	46.0	51.1	48.55
7.	NRCGCS-446	53.4	52.5	52.95
8.	NRCGCS-186	50.0	51.9	50.95
9.	NRCGCS-188	52.0	49.3	50.65
10.	NRCGCS-308	51.0	53.1	52.05
11.	NRCGCS-166	50.0	53.7	51.85
12.	NRCGCS-03	49.8	50.3	50.05
13.	NRCGCS-20	48.5	51.6	50.05
14.	NRCGCS-30	49.5	51.3	51.30
15.	NRCGCS-32	51.8	50.4	51.10
16.	NRCGCS-37	52.0	50.3	51.15
17.	NRCGCS-38	50.0	50.8	50.40
18.	NRCGCS-54	50.7	50.2	50.45
19.	NRCGCS-61	52.0	51.4	51.70
20.	NRCGCS-68	46.5	53.2	49.85
21.	NRCGCS-70	47.3	51.3	49.30
22.	NRCGCS-72	45.0	51.7	48.35
23.	NRCGCS-73	45.5	50.9	48.20
24.	NRCGCS-105	49.0	51.9	50.45
25.	NRCGCS-107	49.0	51.7	50.35
26.	NRCGCS-136	52.5	50.7	51.60
27.	NRCGCS-137	50.0	48.8	49.40
28.	NRCGCS-139	47.0	50.8	48.90
29.	NRCGCS-144	50.5	52.6	51.55
30.	NRCGCS-149	47.5	50.2	48.85
31.	NRCGCS-152	50.5	51.5	51.00
32.	NRCGCS-154	50.0	51.1	50.55
33.	NRCGCS-155	48.0	50.5	49.25
34.	NRCGCS-160	48.0	51.1	49.55
35.	NRCGCS-161	47.0	50.6	48.80
36.	NRCGCS-182	52.0	54.3	53.15
37.	NRCGCS-183	50.5	50.4	50.45
38.	NRCGCS-196	49.0	53.7	51.35
39.	NRCGCS-199	49.5	50.9	50.20
40.	NRCGCS-200	46.5	49.4	47.95
41.	NRCGCS-218	46.7	50.7	48.70
42.	NRCGCS-219	48.0	53.2	50.60
43.	NRCGCS-220	45.5	50.8	48.15

Table 3 continued

S. no.	Genotype	Oil %		
		Kharif, 2017	*Kharif*, 2018	Mean
44.	NRCGCS-234	53.0	51.4	52.20
45.	NRCGCS-239	49.5	50.6	50.05
46.	NRCGCS-245	50.0	52.4	51.20
47.	NRCGCS-246	46.0	52.3	49.15
48.	NRCGCS-252	50.5	49.8	50.15
49.	NRCGCS-256	51.0	54.6	52.80
50.	NRCGCS-257	52.0	51.0	51.50
51.	NRCGCS-260	48.0	50.8	49.40
52.	NRCGCS-264	52.0	49.8	50.90
53.	NRCGCS-265	49.6	49.6	49.60
54.	NRCGCS-266	53.0	49.4	51.20
55.	NRCGCS-269	54.0	49.8	51.90
56.	NRCGCS-270	52.5	51.9	52.20
57.	NRCGCS-280	47.0	52.0	49.50
58.	NRCGCS-281	50.5	53.4	51.95
59.	NRCGCS-282	46.0	49.0	47.50
60.	NRCGCS-285	51.0	51.1	51.05
61.	NRCGCS-303	48.0	51.0	49.50
62.	NRCGCS-311	49.0	51.4	50.20
63.	NRCGCS-315	48.0	51.4	49.70
64.	NRCGCS-319	48.0	49.8	48.90
65.	NRCGCS-327	46.0	48.6	47.30
66.	NRCGCS-349	49.0	49.8	49.40
67.	NRCGCS-385	51.0	51.6	51.30
68.	NRCGCS-409	46.6	51.8	49.20
69.	NRCGCS-411	44.1	54.4	49.25
70.	NRCGCS-412	46.8	53.6	50.20
71.	NRCGCS-414	56.8	55.3	56.05
72.	NRCGCS-417	51.0	50.4	50.70
73.	NRCGCS-424	55.0	53.9	54.45
74.	NRCGCS-426	53.6	54.0	53.80
75.	NRCGCS-432	50.4	51.0	50.70
76.	NRCGCS-434	52.5	49.6	51.05
77.	NRCGCS-461	55.6	53.1	54.35
78.	NRCGCS-475	54.4	53.8	54.10
79.	NRCGCS-492	50.1	52.6	51.35
80.	NRCGCS-532	49.0	51.2	50.10
81.	NRCGCS-536	51.2	50.8	51.00
82.	NRCGCS-430	50.2	49.3	49.75
	Range	39.5–60.0	48.6–55.3	45.4–56.0
	Mean	49.8	51.4	50.6
	CV	4.41		
	CD	4.38		

Table 4 Fatty acid profiles of 82 pre-breeding lines during *kharif*, 2017 and *kharif*, 2018

S. no.	Genotype	Oleic acid %			Linoleic acid %			Palmitic acid %			Oleic acid/Linoleic acid ratio		
		Kharif 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean
1.	NRCGCS-77	51.7	56.0	53.9	27.5	24.3	25.9	11.1	8.7	9.9	1.9	2.3	2.1
2.	NRCGCS-78	53.1	54.7	53.9	26.8	25.0	25.9	10.7	8.9	9.8	2.0	2.2	2.1
3.	NRCGCS-79	51.6	56.0	53.8	27.7	21.9	24.8	11.1	8.8	9.9	1.9	2.6	2.2
4.	NRCGCS-85	50.2	51.6	50.9	28.7	26.7	27.7	11.4	9.1	10.2	1.7	1.9	1.8
5.	NRCGCS-156	50.5	48.1	49.3	26.7	30.3	28.5	12.0	9.8	10.9	1.9	1.6	1.7
6.	NRCGCS-404	40.1	39.3	39.7	35.6	34.7	35.1	13.0	12.1	12.6	1.1	1.1	1.1
7.	NRCGCS-446	46.4	48.6	47.5	30.2	29.2	29.7	12.9	10.4	11.6	1.5	1.7	1.6
8.	NRCGCS-186	51.1	57.2	54.2	27.5	21.4	24.5	11.5	8.4	10.0	1.9	2.7	2.2
9.	NRCGCS-188	58.9	52.4	55.6	19.8	27.7	23.7	9.4	9.4	9.4	3.0	1.9	2.3
10.	NRCGCS-308	45.3	45.0	45.1	31.2	31.0	31.1	12.0	9.4	10.7	1.5	1.4	1.5
11.	NRCGCS-166	50.3	56.6	53.5	26.5	22.1	24.3	12.5	8.6	10.5	1.9	2.6	2.2
12.	NRCGCS-03	50.5	49.4	50.0	27.6	28.8	28.2	12.0	12.3	12.2	1.8	1.7	1.8
13.	NRCGCS-20	51.9	49.1	50.5	26.3	29.2	27.7	11.5	10.1	10.8	2.0	1.7	1.8
14.	NRCGCS-30	50.1	44.8	47.4	28.0	33.1	30.6	11.8	10.8	11.3	1.8	1.4	1.6
15.	NRCGCS-32	44.5	40.0	42.3	33.1	36.9	35.0	12.1	10.9	11.5	1.3	1.1	1.2
16.	NRCGCS-37	40.6	51.1	45.9	36.1	28.7	32.4	13.7	9.5	11.6	1.1	1.8	1.4
17.	NRCGCS-38	51.7	48.2	49.9	28.0	28.7	28.4	11.0	11.0	11.0	1.8	1.7	1.8
18.	NRCGCS-54	50.6	54.2	52.4	26.8	24.7	25.7	12.4	9.6	11.0	1.9	2.2	2.0
19.	NRCGCS-61	54.9	57.7	56.3	23.9	21.6	22.7	10.7	8.5	9.6	2.3	2.7	2.5
20.	NRCGCS-68	59.0	56.5	57.7	20.0	22.7	21.3	9.8	9.5	9.6	3.0	2.5	2.7
21.	NRCGCS-70	55.8	54.4	55.1	23.4	24.5	23.9	10.4	10.3	10.4	2.4	2.2	2.3
22.	NRCGCS-72	54.1	54.2	54.2	24.0	25.3	24.6	11.3	9.0	10.2	2.3	2.1	2.2
23.	NRCGCS-73	40.6	54.7	47.6	36.1	25.3	30.7	12.6	9.0	10.8	1.1	2.2	1.6
24.	NRCGCS-105	50.3	55.1	52.7	28.6	24.8	26.7	11.9	9.6	10.8	1.8	2.2	2.0
25.	NRCGCS-107	51.5	46.5	49.0	27.8	31.3	29.6	11.6	9.4	10.5	1.9	1.5	1.7
26.	NRCGCS-136	47.9	53.9	50.9	30.1	24.9	27.5	11.7	9.1	10.4	1.6	2.2	1.9
27.	NRCGCS-137	48.1	51.8	49.9	28.0	27.2	27.6	12.4	9.3	10.8	1.7	1.9	1.8
28.	NRCGCS-139	57.5	53.1	55.3	23.9	24.7	24.3	10.9	8.9	9.9	2.4	2.2	2.3
29.	NRCGCS-144	51.9	50.7	51.3	26.4	26.8	26.6	11.1	10.4	10.8	2.0	1.9	1.9
30.	NRCGCS-149	49.8	50.4	50.1	27.9	28.0	27.9	12.0	10.5	11.2	1.8	1.8	1.8
31.	NRCGCS-152	52.4	51.9	52.2	26.1	25.5	25.8	11.3	10.7	11.0	2.0	2.0	2.0
32.	NRCGCS-154	52.0	53.6	52.8	25.7	25.2	25.4	12.2	9.6	10.9	2.0	2.1	2.1
33.	NRCGCS-155	53.7	50.2	51.9	25.0	27.7	26.3	10.8	10.2	10.5	2.2	1.8	2.0

Table 4 continued

S. no.	Genotype	Oleic acid %			Linoleic acid %			Palmitic acid %			Oleic acid/Linoleic acid ratio		
		Kharif 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean
34.	NRCGCS-160	50.3	51.2	50.7	28.6	27.5	28.0	11.8	10.4	11.1	1.8	1.9	1.8
35.	NRCGCS-161	52.7	52.4	52.5	25.6	25.5	25.5	11.4	10.1	10.7	2.1	2.1	2.1
36.	NRCGCS-182	58.6	55.3	56.9	20.2	23.0	21.6	9.7	9.0	9.4	2.9	2.4	2.6
37.	NRCGCS-183	54.5	50.3	52.4	24.3	28.5	26.4	10.4	9.8	10.1	2.2	1.8	2.0
38.	NRCGCS-196	44.3	49.9	47.1	32.6	27.6	30.1	13.0	10.4	11.7	1.4	1.8	1.6
39.	NRCGCS-199	49.2	55.1	52.2	27.3	24.1	25.7	12.1	8.7	10.4	1.8	2.3	2.0
40.	NRCGCS-200	48.7	46.7	47.7	29.0	31.3	30.2	11.7	10.7	11.2	1.7	1.5	1.6
41.	NRCGCS-218	50.0	52.9	51.4	29.4	27.0	28.2	11.5	9.4	10.4	1.7	2.0	1.8
42.	NRCGCS-219	53.7	56.3	55.0	24.4	23.0	23.7	11.1	9.8	10.5	2.2	2.4	2.3
43.	NRCGCS-220	55.6	60.0	57.8	23.7	19.9	21.8	10.7	8.4	9.5	2.3	3.0	2.7
44.	NRCGCS-234	52.7	52.1	52.4	26.8	26.8	26.8	10.5	9.6	10.1	2.0	1.9	2.0
45.	NRCGCS-239	45.7	41.5	43.6	32.3	35.2	33.7	12.6	11.9	12.2	1.4	1.2	1.3
46.	NRCGCS-245	48.1	50.5	49.3	30.1	27.3	28.7	12.3	10.6	11.4	1.6	1.8	1.7
47.	NRCGCS-246	52.0	53.2	52.6	26.8	25.7	26.3	11.7	10.4	11.0	1.9	2.1	2.0
48.	NRCGCS-252	51.7	53.1	52.4	26.7	25.5	26.1	11.6	10.3	11.0	1.9	2.1	2.0
49.	NRCGCS-256	50.6	53.9	52.3	28.0	25.3	26.7	12.0	10.1	11.0	1.8	2.1	2.0
50.	NRCGCS-257	40.7	50.3	45.5	36.1	27.8	31.9	13.6	10.6	12.1	1.1	1.8	1.4
51.	NRCGCS-260	50.7	55.4	53.1	26.8	23.4	25.1	12.3	9.2	10.8	1.9	2.4	2.1
52.	NRCGCS-264	50.1	57.2	53.6	29.5	22.6	26.0	11.4	8.7	10.1	1.7	2.5	2.1
53.	NRCGCS-265	53.8	54.2	54.0	24.5	25.1	24.8	11.1	9.1	10.1	2.2	2.2	2.2
54.	NRCGCS-266	51.7	53.0	52.3	26.9	26.9	26.9	11.4	9.0	10.2	1.9	2.0	1.9
55.	NRCGCS-269	50.6	56.6	53.6	28.0	22.5	25.3	12.0	9.8	10.9	1.8	2.5	2.1
56.	NRCGCS-270	48.1	57.6	52.9	30.2	21.6	25.9	11.4	9.3	10.4	1.6	2.7	2.0
57.	NRCGCS-280	44.3	62.3	53.3	33.1	18.2	25.6	12.2	8.6	10.4	1.3	3.4	2.1
58.	NRCGCS-281	51.9	64.5	58.2	26.1	16.1	21.1	11.6	8.1	9.8	2.0	4.0	2.8
59.	NRCGCS-282	55.9	52.5	54.2	23.4	26.4	24.9	10.7	9.9	10.3	2.4	2.0	2.2
60.	NRCGCS-285	49.0	53.9	51.4	30.0	25.7	27.8	11.9	9.4	10.7	1.6	2.1	1.8
61.	NRCGCS-303	54.3	55.3	54.8	24.3	23.9	24.1	9.8	8.4	9.1	2.2	2.3	2.3
62.	NRCGCS-311	54.2	52.9	53.5	24.5	25.5	25.0	9.8	8.9	9.3	2.2	2.1	2.1
63.	NRCGCS-315	45.3	46.6	46.0	33.8	30.8	32.3	12.5	10.4	11.4	1.3	1.5	1.4
64.	NRCGCS-319	53.4	55.8	54.6	26.1	24.6	25.3	10.9	9.2	10.0	2.0	2.3	2.2
65.	NRCGCS-327	48.8	52.4	50.6	28.9	26.5	27.7	11.7	10.6	11.2	1.7	2.0	1.8
66.	NRCGCS-349	55.9	56.9	56.4	23.2	22.8	23.0	10.6	10.7	10.6	2.4	2.5	2.5

Table 4 continued

S. no.	Genotype	Oleic acid %			Linoleic acid %			Palmitic acid %			Oleic acid/Linoleic acid ratio		
		Kharif 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean
67.	NRCGCS-385	58.5	61.6	60.1	22.0	18.6	20.3	9.9	8.8	9.4	2.7	3.3	3.0
68.	NRCGCS-409	60.8	62.0	61.4	19.2	20.9	20.0	9.9	9.5	9.7	3.2	3.0	3.1
69.	NRCGCS-411	63.3	61.9	62.6	17.1	18.5	17.8	9.3	8.2	8.8	3.7	3.4	3.5
70.	NRCGCS-412	61.1	60.3	60.7	19.1	19.8	19.5	10.2	9.2	9.7	3.2	3.0	3.1
71.	NRCGCS-414	59.5	63.4	61.5	20.6	17.2	18.9	10.3	8.1	9.2	2.9	3.7	3.3
72.	NRCGCS-417	56.6	56.2	56.4	22.9	22.9	22.9	10.9	9.5	10.2	2.5	2.5	2.5
73.	NRCGCS-424	59.8	64.4	62.1	20.1	16.1	18.1	10.4	8.5	9.4	3.0	4.0	3.4
74.	NRCGCS-426	55.7	61.7	58.7	23.1	18.8	21.0	10.6	8.3	9.4	2.4	3.3	2.8
75.	NRCGCS-432	55.7	60.3	58.0	23.3	19.6	21.4	11.1	8.9	10.0	2.4	3.1	2.7
76.	NRCGCS-434	56.7	55.3	56.0	23.2	23.8	23.5	10.6	9.0	9.8	2.4	2.3	2.4
77.	NRCGCS-461	61.2	64.2	62.7	18.9	16.4	17.7	10.2	8.6	9.4	3.2	3.9	3.5
78.	NRCGCS-475	40.9	46.2	43.6	34.7	30.3	32.5	14.2	11.0	12.6	1.2	1.5	1.3
79.	NRCGCS-492	59.1	61.9	60.5	20.7	18.6	19.6	10.5	8.2	9.3	2.9	3.3	3.1
80.	NRCGCS-532	55.9	57.6	56.8	23.4	21.7	22.6	10.7	8.3	9.5	2.4	2.7	2.5
81.	NRCGCS-536	54.0	55.7	54.8	24.5	22.4	23.4	11.0	8.7	9.9	2.2	2.5	2.3
82.	NRCGCS-430	56.1	60.4	58.2	23.4	19.8	21.6	10.7	9.0	9.8	2.4	3.0	2.7
	Range	40.1 to 63.3	39.3 to 64.5	39.7 to 62.7	17.0 to 36.1	16.0 to 36.9	17.7 to 35.1	9.3 to 14.2	8.1 to 12.3	8.7 to 12.6	1.1 to 3.7	1.0 to 4.0	1.1 to 3.5
	Mean	51.96	53.92	52.94	26.57	24.99	25.8	11.36	9.55	10.46	2.04	2.26	2.13
	CV	6.22			5.25			1.39			0.72		
	CD	5.90			10.24			6.67			16.73		

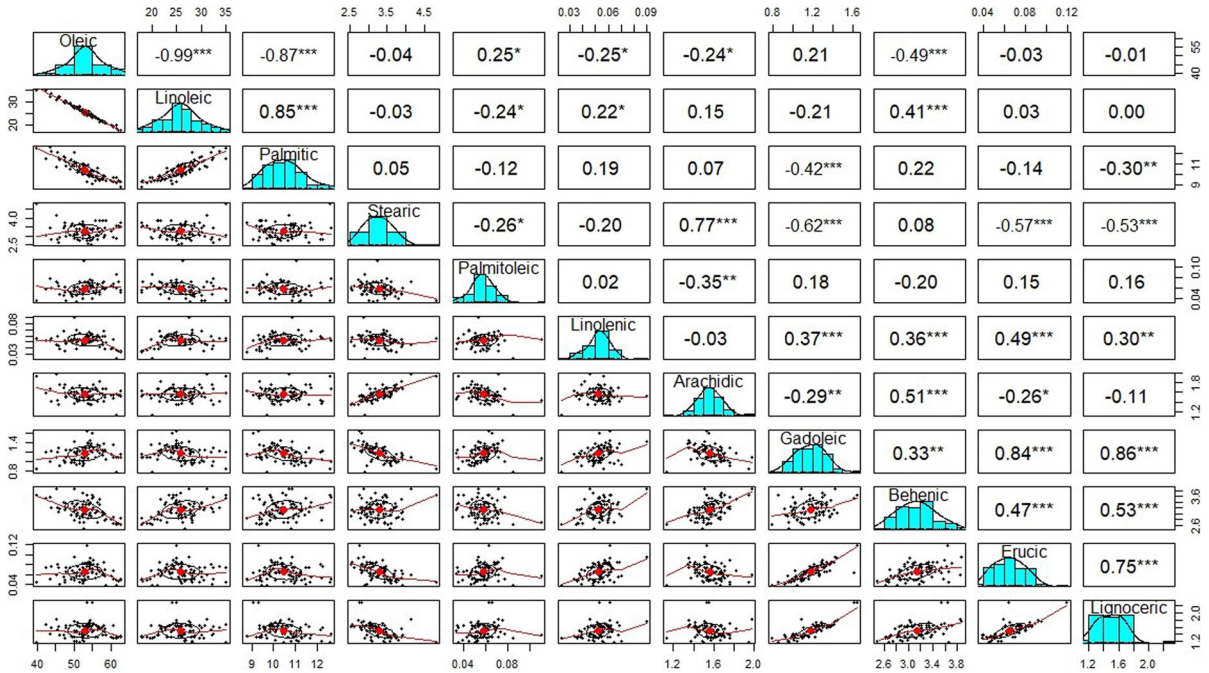

Fig. 1 Correlation coefficients among fatty acids

rose-colored testa, apart from a few lines that had dark red or variegated testa (Fig. 3).

Late leaf spot incidence

Late leaf spot (LLS) is one of the major foliar fungal disease in groundnut. The selected 82 PBLs were characterized for resistance to LLS. In PBLs, LLS incidence was comparatively low in the year 2017 than in 2018. LLS scores also varied from 3 to 9, indicating very high disease incidence in inoculated field conditions in both the years. None of the breeding lines were found resistant to LLS; however, 21 lines were found moderately resistant with a 3–5 disease score (Table 5).

Superior pre-breeding lines

PBLs with multiple desirable traits would be a better donor for groundnut improvement. NRCGCS-409, NRCGCS-412, NRCGCS-414, and NRCGCS-424 had high oleic acid content, while NRCGCS-385, NRCGCS-409, NRCGCS-411, NRCGCS-412, NRCGCS-414, NRCGCS-424, NRCGCS-461 NRCGCS-492 had O/L ratio more than 3. Similarly, NRCGCS-414, NRCGCS-424, NRCGCS-426, NRCGCS-461, and NRCGCS-475 had very high oil content (> 52%). High pod yield and HKW were observed in NRCGCS-281, NRCGCS-285, NRCGCS-385, NRCGCS-409, and NRCGCS-412. Earliness was reported in NRCGCS-199, NRCGCS-260, and NRCGCS-404 (<20 days DFF). However moderate level of resistance to LLS was observed in NRCGCS-03, NRCGCS-38, NRCGCS-73, NRCGCS-77, NRCGCS-78, NRCGCS-79, NRCGCS-85, NRCGCS-136, NRCGCS-137, NRCGCS-139, NRCGCS-156, NRCGCS-166, NRCGCS-186, NRCGCS-188, NRCGCS-265, NRCGCS-303, NRCGCS-311, NRCGCS-430, NRCGCS-432, and NRCGCS-434. Thus, NRCGCS-414 and NRCGCS-424 had higher oil content, oleic acid content, and O/L ratio while NRCGCS-412 had higher oleic acid content, O/L ratio, pod yield and HKW (Table 6).

Discussion

Across the globe for the last 2 decades, there is a great increase in the use of peanut for confectionary and

Table 5 Disease reaction and yield traits of the 82 pre-breeding lines in *kharif*, 2017 and *kharif*, 2018

S. no.	Genotypes	Pod wt./10 plant (g)			HKW (g)			Earliness (days after germination)			LLS score (1–9 scale)			Testa color	
		Kharif 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018
1	NRCGCS-03	66.0	66.0	66.0	30.0	32.0	31.0	21	21	21	0	4	4	Rose	Rose
2	NRCGCS-20	50.0	53.0	51.5	48.0	47.0	47.5	21	21	21	6	5	6	Rose	Rose
3	NRCGCS-30	70.0	70.0	70.0	30.0	31.0	30.5	23	24	24	6	5	6	Rose	Rose
4	NRCGCS-32	59.0	60.0	59.5	34.0	32.0	33.0	21	21	21	7	7	7	Rose	Rose
5	NRCGCS-37	100.0	102.0	101.0	36.0	36.0	36.0	22	22	22	7	7	7	Rose	Rose
6	NRCGCS-38	77.0	75.0	76.0	36.0	34.0	35.0	22	22	22	4	3	4	Rose	Rose
7	NRCGCS-54	83.0	80.0	81.5	26.0	25.0	25.5	21	21	21	7	8	8	Rose	Rose
8	NRCGCS-61	67.0	65.0	66.0	30.0	32.0	31.0	21	21	21	6	5	6	Rose	Rose
9	NRCGCS-68	85.0	88.0	86.5	46.0	46.0	46.0	23	24	24	7	7	7	Rose	Rose
10	NRCGCS-70	71.0	75.0	73.0	38.0	35.0	36.5	23	24	24	6	5	6	Rose	Rose
11	NRCGCS-72	47.0	50.0	48.5	42.0	40.0	41.0	23	23	23	6	5	6	Rose	Rose
12	NRCGCS-73	38.0	45.0	41.5	38.0	36.0	37.0	23	24	24	3	3	3	Rose	Rose
13	NRCGCS-77	57.0	55.0	56.0	38.0	36.0	37.0	23	25	24	2	3	3	Rose	Rose
14	NRCGCS-78	66.0	65.0	65.5	34.0	35.0	34.5	22	24	23	2	3	3	Rose	Rose
15	NRCGCS-79	66.0	66.0	66.0	40.0	40.0	40.0	23	25	24	2	3	3	Rose	Rose
16	NRCGCS-85	65.0	67.0	66.0	42.0	41.0	41.5	22	22	22	2	3	3	Rose	Rose
17	NRCGCS-105	65.0	60.0	62.5	36.0	38.0	37.0	21	22	22	7	7	7	Rose	Rose
18	NRCGCS-107	100.0	96.0	98.0	42.0	45.0	43.5	21	22	22	7	7	7	Rose	Rose
19	NRCGCS-136	60.0	64.0	62.0	36.0	36.0	36.0	24	24	24	4	4	4	Rose	Rose
20	NRCGCS-137	51.0	55.0	53.0	50.0	49.0	49.5	22	24	23	3	3	3	Rose	Rose
21	NRCGCS-139	66.0	70.0	68.0	34.0	35.0	34.5	19	24	22	4	3	4	Rose	Rose
22	NRCGCS-144	73.0	68.0	70.5	40.0	40.0	40.0	21	21	21	5	3	5	Rose	Rose
23	NRCGCS-149	68.0	70.0	69.0	44.0	44.0	44.0	19	23	21	5	3	5	Rose	Rose
24	NRCGCS-152	55.0	60.0	57.5	32.0	32.0	32.0	22	23	23	6	5	6	Rose	Rose
25	NRCGCS-154	102.0	97.0	99.5	30.0	32.0	31.0	22	23	23	6	5	6	Rose	Rose
26	NRCGCS-155	57.0	61.0	59.0	38.0	40.0	39.0	19	24	22	5	6	6	Rose	Rose
27	NRCGCS-156	85.0	88.0	86.5	34.0	35.0	34.5	22	23	23	3	3	3	Rose	Rose
28	NRCGCS-160	71.0	66.0	68.5	24.0	28.0	26.0	22	24	23	5	6	6	Rose	Rose
29	NRCGCS-161	77.0	73.0	75.0	40.0	42.0	41.0	22	22	22	5	6	6	Rose	Rose
30	NRCGCS-166	74.0	75.0	74.5	26.0	25.0	25.5	22	23	23	4	4	4	Rose	Rose
31	NRCGCS-182	63.0	65.0	64.0	56.0	57.0	56.5	23	24	24	5	3	5	Rose	Rose

Table 5 continued

S. no.	Genotypes	Pod wt./10 plant (g)			HKW (g)			Earliness (days after germination)			LLS score (1–9 scale)			Testa color	
		Kharif 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018
32	NRCGCS-183	42.0	45.0	43.5	48.0	49.0	48.5	23	24	24	6	5	6	Rose	Rose
33	NRCGCS-186	72.0	72.0	72.0	46.0	45.0	45.5	25	24	25	3	3	3	Rose	Rose
34	NRCGCS-188	81.0	80.0	80.5	52.0	50.0	51.0	23	23	23	3	3	3	Rose	Rose
35	NRCGCS-196	58.0	55.0	56.5	28.0	30.0	29.0	22	22	22	5	5	5	Rose	Rose
36	NRCGCS-199	67.0	65.0	66.0	52.0	50.0	51.0	16	16	16	5	5	5	Rose	Rose
37	NRCGCS-200	61.0	60.0	60.5	34.0	35.0	34.5	23	22	23	6	6	6	Rose	Rose
38	NRCGCS-218	90.0	88.0	89.0	30.0	33.0	31.5	22	22	22	6	5	6	Rose	Rose
39	NRCGCS-219	74.0	80.0	77.0	48.0	45.0	46.5	23	24	24	4	5	5	Rose	Rose
40	NRCGCS-220	33.0	40.0	36.5	40.0	42.0	41.0	23	24	24	4	5	5	Rose	Rose
41	NRCGCS-234	63.0	68.0	65.5	36.0	38.0	37.0	27	23	25	6	5	6	Rose	Rose
42	NRCGCS-239	98.0	95.0	96.5	34.0	35.0	34.5	23	23	23	7	5	7	Rose	Rose
43	NRCGCS-245	61.0	60.0	60.5	34.0	36.0	35.0	21	22	22	5	5	5	Rose	Rose
44	NRCGCS-246	50.0	49.0	49.5	40.0	39.0	39.5	22	24	23	4	3	4	Rose	Rose
45	NRCGCS-252	81.0	80.0	80.5	48.0	48.0	48.0	23	23	23	4	5	5	Rose	Rose
46	NRCGCS-256	71.0	77.0	74.0	56.0	55.0	55.5	23	22	23	4	5	5	Rose	Rose
47	NRCGCS-257	56.0	64.0	60.0	28.0	30.0	29.0	21	22	22	4	5	5	Rose	Rose
48	NRCGCS-260	58.0	60.0	59.0	28.0	32.0	30.0	18	19	19	5	5	5	Rose	Rose
49	NRCGCS-264	92.0	86.0	89.0	52.0	50.0	51.0	23	23	23	5	5	5	Rose	Rose
50	NRCGCS-265	71.0	73.0	72.0	42.0	42.0	42.0	24	23	24	4	4	4	Rose	Rose
51	NRCGCS-266	80.0	77.0	78.5	48.0	48.0	48.0	23	24	24	5	5	5	Rose	Rose
52	NRCGCS-269	95.0	99.0	97.0	56.0	56.0	56.0	21	22	22	5	5	5	Rose	Rose
53	NRCGCS-270	100.0	96.0	98.0	40.0	39.0	39.5	23	23	23	5	5	5	Rose	Rose
54	NRCGCS-280	63.0	66.0	64.5	48.0	45.0	46.5	22	22	22	4	5	5	Rose	Rose
55	NRCGCS-281	115.0	110.0	112.5	84.0	82.0	83.0	21	20	21	4	5	5	Rose	Rose
56	NRCGCS-282	45.0	50.0	47.5	32.0	35.0	33.5	23	23	23	4	5	5	Variegated	Variegated
57	NRCGCS-285	100.0	110.0	105.0	56.0	55.0	55.5	21	21	21	5	5	5	Rose	Rose
58	NRCGCS-303	45.0	52.0	48.5	44.0	43.0	43.5	23	24	24	4	4	4	Variegated	Variegated
59	NRCGCS-308	45.0	43.0	44.0	24.0	25.0	24.5	22	21	22	7	7	7	Dark red	Dark red
60	NRCGCS-311	71.0	73.0	72.0	40.0	42.0	41.0	22	23	23	4	3	4	Variegated	Variegated
61	NRCGCS-315	78.0	75.0	76.5	38.0	38.0	38.0	21	21	21	6	5	6	Rose	Rose
62	NRCGCS-319	73.0	70.0	71.5	42.0	40.0	41.0	25	26	26	5	5	5	Rose	Rose

Table 5 continued

S. no.	Genotypes	Pod wt./10 plant (g)			HKW (g)			Earliness (days after germination)			LLS score (1–9 scale)			Testa color	
		Kharif 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018	Mean	*Kharif* 2017	*Kharif* 2018
63	NRCGCS-327	53.0	60.0	56.5	34.0	34.0	34.0	21	22	22	6	5	6	Rose	Rose
64	NRCGCS-349	59.0	65.0	62.0	40.0	44.0	42.0	24	23	24	5	5	5	Rose	Rose
65	NRCGCS-385	99.0	105.0	102.0	54.0	56.0	55.0	23	23	23	8	8	8	Rose	Rose
66	NRCGCS-404	70.0	73.0	71.5	32.0	32.0	32.0	19	18	19	8	8	8	Rose	Rose
67	NRCGCS-409	118.0	114.0	116.0	56.0	53.0	54.5	23	22	23	8	9	9	Rose	Rose
68	NRCGCS-411	96.0	100.0	98.0	58.0	59.0	58.5	22	22	22	6	7	7	Rose	Rose
69	NRCGCS-412	106.0	110.0	108.0	52.0	53.0	52.5	24	23	24	6	7	7	Rose	Rose
70	NRCGCS-414	81.0	88.0	84.5	54.0	54.0	54.0	24	22	23	6	6	6	Rose	Rose
71	NRCGCS-417	68.0	73.0	70.5	28.0	32.0	30.0	24	22	23	5	5	5	Rose	Rose
72	NRCGCS-424	70.0	80.0	75.0	58.0	59.0	58.5	21	21	21	4	5	5	Rose	Rose
73	NRCGCS-426	58.0	49.0	53.5	52.0	50.0	51.0	21	21	21	5	5	5	Rose	Rose
74	NRCGCS-430	45.0	50.0	47.5	36.0	35.0	35.5	24	23	24	4	4	4	Rose	Rose
75	NRCGCS-432	67.0	67.0	67.0	40.0	44.0	42.0	23	24	24	4	4	4	Rose	Rose
76	NRCGCS-434	58.0	63.0	60.5	34.0	32.0	33.0	23	25	24	4	4	4	Rose	Rose
77	NRCGCS-446	70.0	69.0	69.5	52.0	50.0	51.0	20	19	20	6	7	7	Rose	Rose
78	NRCGCS-461	90.0	89.0	89.5	54.0	54.0	54.0	21	21	21	5	5	5	Rose	Rose
79	NRCGCS-475	14.0	20.0	17.0	16.0	23.0	19.5	25	25	25	5	5	5	Rose	Rose
80	NRCGCS-492	23.0	30.0	26.5	50.0	50.0	50.0	21	22	22	6	5	6	Rose	Rose
81	NRCGCS-532	20.0	23.0	21.5	22.0	25.0	23.5	24	23	24	5	5	5	Rose	Rose
82	NRCGCS-536	28.0	35.0	31.5	24.0	27.0	25.5	24	23	24	5	3	5	Rose	Rose
	Mean	68.5	69.9	69.2	40.5	40.9	40.7	22.2	22.5	22.4	5.0	4.9	5.3	–	–
	Min	14.0	20.0	17.0	16.0	23.0	19.5	16.0	16.0	16.0	0.0	3.0	3.0	–	–
	Max	118.0	114.0	116.0	84.0	82.0	83.0	27.0	26.0	25.5	8.0	9.0	9.0	–	–
	CD	3.68	3.57	3.59	3.09	3.03	3.06	1.92	1.91	1.91	1.84	1.84	1.82	–	–
	CV	32.3	28.0	29.0	27.1	24.8	25.8	7.4	7.1	7.3	27.7	28.4	25.3	–	–

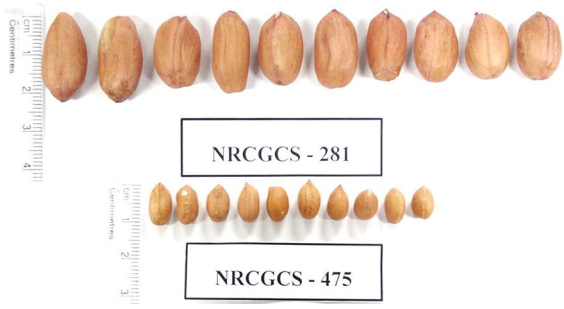

Fig. 2 Variation in kernel size

food purposes rather than the extraction of oil. Previously, peanut varieties with high (49–52%) or very high (> 52%) oil content were in high demand because of the greater yield resulting in higher return compared to the low oil content variety. Recently, the focus has shifted toward the quality of the oil. The fatty acid profile determines the quality and shelf-life of peanut oil and peanut-based foods (Barkely et al. 2013). Hence, improving the oil content and seed fatty acid composition are two important objectives in peanut breeding worldwide.

Peanut oils with high oleic acid have multiple health benefits, including decreasing the cholesterol levels and the lower risk of CVD. On the other hand, low linoleic acid content reduces the risk of tumor genesis and improves the stability of the peanut oil by reducing oxidation and delaying rancidity (Janila et al. 2016). Moreover, higher oleic to linoleic acid ratio elevates the shelf-life of peanut oil since oleic acid is less prone to oxidation. Therefore, there is a massive demand for high oleic peanuts.

The success of any crop-improvement program lies on the availability of important and diverse donor sources in a conventionally usable form. So far, SunOleic 95R or its sister lines or breeding lines developed from SunOleic 95R have been used

Fig. 3 Variation in testa color

extensively across the globe by introgression of high oleic traits in a different background, either through conventional or molecular breeding. Breeding peanut genotypes with > 70% oleic acid requires mutation in both *ahFAD2A* and *ahFAD2B* alleles. Mutation in either allele restricts oleic acid accumulation, maximum up to ~ 65%. Previous research has confirmed the availability of alternative sources of *ahFAD2A* mutant allele across the world in a range of genotypes, with maximum oleic acid content up to 65%. More precisely, to date, no alternative source of *ahFAD2B* mutant allele is available other than SunOleic 95R. This led us to search for additional sources of *ahFAD2A* and *ahFAD2B* mutant alleles in about 563 PBLs. Although several PBLs were identified positive

Table 6 Promising pre-breeding lines with *ahFAD2A* mutant allele and other desirable traits

Traits	Genotype
Oleic acid (%) (≥ 60%)	NRCGCS nos.: 385, 409, 411, 412, 414, 424, 461, 492
Oil content (%) (≥ 55%)	NRCGCS nos.: 79, 414
Late leaf spot score (< 5)	NRCGCS nos.: 3, 38, 73, 77,78,79,85, 136, 137, 139, 156, 166, 186, 188, 246, 265, 303, 311, 430, 432, 434
100 kernel weight (g) (> 50 g)	NRCGCS nos.: 182, 188, 199, 256, 264, 269, 281, 285, 385, 409, 411, 412, 414, 424, 426, 446, 461
Pod weight/10 plant (g) (≥ 100 g)	NRCGCS nos.: 37, 281, 285, 385, 409, 412

for *ahFAD2A* mutant allele, they were negative for *ahFAD2B* mutant allele. Similarly, previous attempts for identifying the alternate source of *ahFAD2B* mutant allele also remained unsuccessful (Chu et al. 2007; Wang et al. 2011, 2013; Nawade et al. 2016; Bera et al. 2018b). Thus, *ahFAD2B* mutant allele available in the F435 breeding line developed at the Florida Experimental Agriculture Station, USA, is novel in this direction.

We also report the availability of new sources for *ahFAD2A* mutant allele with oleic acid content up to 63%. The availability of *ahFAD2A* mutant allele in peanuts with diverse origins across the continents needs further research, although different views have been expressed. Wang et al. (2010) was in opinion that *ahFAD2* gene mutation might have occurred after the polyploidization event in cultivated peanut. In such case, *ahFAD2A* mutant allele should not be present in diploid *Arachis* species. However, Nawade et al. (2016) indicated that *ahFAD2A* mutant allele is also frequently available among the diploid *Arachis* species. These wild species have been collected from their center of origin and maintained at different institutes across the world (Liao 2017). Thus, the observation of Wang et al. (2010) on the origin of *ahFAD2A* mutant allele in *Arachis* genome is incorrect.

On the other hand, Nawade et al. (2016) anticipated that *ahFAD2A* gene mutation could have occurred before the global distribution of peanut from its center of origin and thus, being more frequent in peanut genotypes. In our studies, we identified *ahFAD2A* mutant allele in two GG2 mutants developed through EMS treatment, namely, NRCGCS-385 and NRCGCS-404, but absent in cultivar GG-2. Thus, the observation of Nawade et al. (2016) on the origin of *ahFAD2A* mutant allele in *Arachis* genome is also incorrect. It seems that mutation in *ahFAD2A* locus is a frequent event in the *Arachis* genome, irrespective of genome evolution and migration of the *Arachis* species. The locus could be more amenable to mutation by both physical and chemical mutagens, although frequency lies on the genotypes, as well as the type and dosage of mutagens (unpublished data, Bera; Mondal et al. 2011). However, a mutation in *ahFAD2A* allele does not always guaranty a higher accumulation of oleic acid. There was a 36% increase in oleic acid in NRCGCS-385 compared to GG 2, while no increase in NRCGCS-404 (39.7%).

Furthermore, from the analysis of botanical peanut types, we find more frequent *ahFAD2A* mutant allele in Virginia compared to Spanish types. NRCGCS-385 is a VB genotype, while NRCGCS-404 and GG2 are SB genotypes. The growth habit of GG2 changed from SB to VB in NRCGCS-385 and showed an elevation in oleic acid content. However, *ahFAD2A* mutant allele was absent in other VB mutants of GG2, which had normal oleic acid content (Table 7). This probably ascertains the null association between changes in the growth habit and induction of mutation in *ahFAD2A* allele. Findings of Nawade et al. (2016) also clearly demonstrate no correlation between habit group and presence of *ahFAD2A* allele as well as habit group and oleic acid content. We observed a higher frequency of *ahFAD2A* mutant alleles in VR and VB genotypes than SB genotypes. A similar trend was also reflected in the studies of Nawade et al. (2016). Most likely, VB/VR genotypes are more amenable to G-A

Table 7 Details of Virginia Bunch mutants of cultivar GG 2

Mutant lines	Pedigree	Growth habit	*ahFAD2A* mutant allele	Oleic acid (%)	Linoleic acid (%)	Palmitic acid (%)	Oleic/linoleic ratio
NRCGCS-376	GG 2 mutant	VB	Absent	37.6	38.0	14.0	0.98
NRCGCS-377	GG 2 mutant	VB	Absent	43.0	32.0	12.6	1.34
NRCGCS-390	GG 2 mutant	VB	Absent	43.1	33.7	11.9	1.27
NRCGCS-393	GG 2 mutant	VB	Absent	39.0	35.0	12.9	1.11
NRCGCS-402	GG 2 mutant	VB	Absent	38.7	37.8	12.3	1.02
NRCGCS-385	GG 2 mutant	VB	Present	60.1	20.3	9.4	3.0
NRCGCS-404	GG 2 mutant	SB	Present	39.7	35.1	12.6	1.1
GG 2	J 11 × EC-16659	SB	Absent	42.0	39.5	14.8	1.1

substitution mutation than SB genotypes. Alternatively, in majority cases, genes controlling growth habit and *ahFAD2A* gene mutate simultaneously. Most of the high oleic peanut varieties have either VB or VR growth habit with longer duration (Mondal et al. 2018). In our study, maximum oleic acid (62.70%) and O/L ratio (3.5) were observed in Virginia bunch PBLs.

In peanuts, fatty-acid compositions were controlled by genetic factors and their interaction with the environment (Andersen and Gorbet 2002; Singkham et al. 2010) and temperature (Sun et al. 2014). However, in our study, we observed stable expression of oleic acid over seasons, indicating minimal or no environmental influence on high oleic acid trait. This illustrates that this trait might be controlled by a limited number of major genes. A strong association between traits helps in improving several traits, simultaneously. Highly negative correlation ($r = -0.990$) was observed between two major fatty acids, namely, oleic acid and linoleic acid, since oleic acid catalyzes into linoleic acid.

Similarly, a strong negative association between oleic acid and palmitic acid ($r = -0.867$) was also seen. Thus, this would help in manipulating these three fatty acids simultaneously. As observed in this study, other reports also revealed *ahFAD* mutant alleles controlling three major fatty acids viz. Oleic, linoleic, and palmitic acid (Pandey et al. 2014; Janila et al. 2016). Additionally, a positive association between SFAs would also be beneficial to target multiple fatty acids together. The PBLs with *ahFAD2A* mutant allele, resistance to LLS, having a short duration, higher pod yield and other yield-related traits identified in this study, could serve as useful donors for introgression of several traits together in cultivated type, without many resources, effort and time (Bera et al. 2018a). Moreover, these PBLs with multiple desirable traits could thus be used for the induction of new *ahFAD2B* mutant allele through mutation breeding or translational genomics research for achieving higher genetic gains in groundnut (Ojiewo et al. 2020; Pandey et al. 2020).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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