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Molecular breeding for introgression of fatty acid desaturase mutant alleles (*ahFAD2A* and *ahFAD2B*) enhances oil quality in high and low oil containing peanut genotypes

Pasupuleti Janila[a,1], Manish K Pandey[a,1], Yaduru Shasidhar[a], Murali T Variath[a], Manda Sriswathi[a], Pawan Khera[a], Surendra S Manohar[a], Patne Nagesh[a], Manish K Vishwakarma[a], Gyan P Mishra[b], T Radhakrishnan[b], N Manivannan[c], KL Dobariya[d], RP Vasanthi[e], Rajeev K Varshney[a,*]

[a] *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India*
[b] *Directorate of Groundnut Research (DGR) of Indian Council of Agricultural Research (ICAR), Junagadh, India*
[c] *Department of Oilseeds, Centre of Plant Breeding and Genetics, Tamil Nadu Agricultural University (TNAU), Coimbatore, India*
[d] *Main Oilseeds Research Station, Junagadh Agricultural University (JAU), Junagadh, India*
[e] *Regional Agricultural Research Station, Acharya NG Ranga Agricultural University (ANGRAU), Tirupati, India*

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ABSTRACT

High oleate peanuts have two marketable benefits, health benefits to consumers and extended shelf life of peanut products. Two mutant alleles present on linkage group a09 (*ahFAD2A*) and b09 (*ahFAD2B*) control composition of three major fatty acids, oleic, linoleic and palmitic acids which together determine peanut oil quality. In conventional breeding, selection for fatty acid composition is delayed to advanced generations. However by using DNA markers, breeders can reject large number of plants in early generations and therefore can optimize time and resources. Here, two approaches of molecular breeding namely marker-assisted backcrossing (MABC) and marker-assisted selection (MAS) were employed to transfer two *FAD2* mutant alleles from SunOleic 95R into the genetic background of ICGV 06110, ICGV 06142 and ICGV 06420. In summary, 82 MABC and 387 MAS derived introgression lines (ILs) were developed using DNA markers with elevated oleic acid varying from 62 to 83%. Oleic acid increased by 0.5–1.1 folds, with concomitant reduction of linoleic acid by 0.4–1.0 folds and palmitic acid by 0.1–0.6 folds among ILs compared to recurrent parents. Finally, high oleate ILs, 27 with high oil (53–58%), and 28 ILs with low oil content (42–50%) were selected that may be released for cultivation upon further evaluation.

1. Introduction

Peanut or groundnut (*Arachis hypogaea* L.) is one of the major oil and food crops of the world. It is cultivated in >100 countries in an area of 25.44 m ha with total production of 45.22 m tons [1]. China, India, Nigeria and United States of America are the leading producers with ~70% contribution to the global peanut production. It is an important cash crop for the farmers of the arid and semi-arid tropics, where most of the peanut cultivation is concentrated. All parts of the crop are useful but the most important part is the seed which is used for oil extraction, eaten as fresh/boiled/roasted, used in preparation of several confectionary items, flour, cake and butter. The inferior quality oil is used for making soaps, detergents, cosmetics, paints, candles and lubricants. Ready-to-use-therapeutic products made from peanuts are commonly used to treat acute malnutrition among children, women and patients by UNICEF in several countries of Africa and Asia. Besides seeds, the above ground plant parts constitute nutritious fodder for livestock. The by-products such as peanut shells are useful as fuel source, as filler for making particle boards and as animal feed. Being a legume crop, peanut also contributes towards mak-

Abbreviations: MABC, marker-assisted backcrossing; MAS, marker-assisted selection; ILs, introgression lines; CVD, cardiovascular diseases; LDL, low density lipoproteins; HDL, high density lipoproteins; FAD, fatty acid desaturase; CAPS, cleaved amplified polymorphic sequences; AS-PCR, allele specific polymerase chain reaction; ICGV, ICRISAT groundnut variety.

* Corresponding author. Fax: +91 4030713074.
E-mail address: r.k.varshney@cgiar.org (R.K. Varshney).
[1] These authors contributed equally.

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ing the soil healthy and fertile through biological nitrogen fixation. Although peanut is known for being a major source of fat, it is also a good source for protein (~25%), micronutrients (minerals, antioxidants and vitamins) and secondary metabolites (flavonoid, folic acid, tocopherols and resveratrol) [2,3].

Diverse preferences for peanut seed quality and fatty acid composition including extreme level of oil content exist in the society and industry. This diversity is driven by regional choices which largely influence the market and peanut food processing industry. For example, the regional preferences exist for high as well as low oil content. In China and India, majority of peanuts are crushed for oil extraction and the preference is for high oil content, while in USA and European countries, it is mostly used for confectionary and other food uses [4]. Low oil content peanuts are preferred for table purposes and food preparations with low calorific value. However, for both low and high oil containing peanuts, good oil quality is an important preference to meet the needs of the consumers and industry. Oil quality is determined by the fatty acid composition in the seeds. In peanut, two unsaturated fatty acids (UFA) namely, oleic acid, a monounsaturated UFA (MUFA) and linoleic acid, a polyunsaturated UFA (PUFA), together constitute up to 80% of the peanut oil. The remaining 20% of total fatty acid includes six saturated fatty acids (SFA) with palmitic acid alone contributing to about 10%, and stearic, arachidic, gadoleic, behenic, and lignoceric acids together making up the remaining 10% [5]. The oleic, linoleic and palmitic acids are the three major fatty acids in peanut oil, and together they constitute ~90% of the total fatty acid composition, thus the composition of these three major fatty acid determines the oil quality in peanut [6].

The peanut oil is one of the healthy cooking oil as the ratio of UFAs to SFAs in peanut oil is very high as compared to coconut and palm oil [7]. In general, SFAs are considered to increase serum low-density lipoproteins (LDL) cholesterol level. Palmitic acid is known to cause adverse effect on human health as it has been found to increase the risk of developing cardiovascular diseases (CVD) [8]. The high amount of linoleic acid (PUFA) in the oil is not good for cooking purposes as it is vulnerable to oxidative rancidity and becomes thermodynamically unstable when heated at high temperature [9]. Furthermore, such instability in linoleic acid leads to formation of *trans* fatty acid and can cause CVD. In contrast to detrimental effects of high consumption of oil rich in linoleic and palmitic acids on human health, the high oleic acid in a cooking oil offers several health benefits such as decreasing the risk of CVD by reducing the levels of serum LDL cholesterol and maintaining the levels of high-density lipoproteins (HDL) [10,11,12]. Health benefits of diet rich in oleic acid also include suppression of tumorigenesis, and amelioration of inflammatory diseases [13,14]. Oleic acid has 10-fold higher auto-oxidative stability than linoleic acid [15] and therefore, with high oleic to linoleic acid ratio (O/L ratio), peanut and its products have longer shelf life than normal lines [16]. Peanut seeds of a genotype with high O/L ratio have longer shelf life compared to low O/L ratio seeds [17]. Besides, the salted and roasted peanuts made using peanuts with high oleic acid content had longer shelf life [18]. Therefore, breeding improved lines with high oleic acid and low linoleic and palmitic acids in the peanut oil is required to make it healthier for consumers [19]. Besides, the food products made from high oleic peanuts enhanced shelf life as oleic acid is less prone to oxidation. Therefore, for both oil and food processing industry, it is a high priority to introduce high oleate trait into peanut cultivars to provide extended shelf life to peanuts and its products. It is very important to breed high oleate peanut cultivars in Asia and Africa to enhance livelihoods of small and marginal farmers, as well as benefit all stakeholders of the value chain.

The "International Peanut Genome Initiative (IPGI)" has recently decoded the genomes of two diploid progenitors representing A-genome (*Arachis duranensis*, accession V14167) and B-genome (*Arachis ipaensis*, accession K30076). The genome size for *A. duranensis* and *A. ipaensis* was found to be 1.1 and 1.38 Gb, respectively (http://peanutbase.org/genomes). In addition to above, the draft genome sequence for *A. duranensis* (accession PI475845) was also completed by "Diploid Progenitor Peanut A-genome Sequencing Consortium (DPPAGSC)" which further revealed a genome size of 1.07 Gb. Since the detailed genome analysis of these draft genomes are still unpublished, the details on synteny to other legume and oilseeds crops is very limited. The availability of these sequences will accelerate gene discovery and its deployment in routine peanut breeding program. Nevertheless in peanut, a mutant line, F435 with 80% oleic acid and 2% linoleic acid was reported and since its discovery, it was possible to enhance oleic acid content in peanuts [19]. Following conventional breeding methods, the first ever high oleate peanut line, SunOleic 95R was bred in USA [20]. Subsequently, associated markers to the mutant alleles were developed and marker-assisted backcrossing (MABC) was used to improve oleic acid content of a nematode resistant variety, 'Tifguard', and an improved breeding line 'Tifguard High O/L' was developed [21]. In conventional breeding, selection for fatty acid composition is carried out in advance generations, thus requires huge resources to handle and advance large populations till selections are exercised. However, it is possible to reject large number of plants in early generations with use of makers associated with *ahFAD2* mutant alleles, thus optimizing resources and time.

Molecular breeding offers great opportunity for accelerated development of improved cultivars with high precision and accuracy [22,23]. The earlier studies showed that two mutant fatty acid desaturase (*ahFAD*) alleles in the A-genome (linkage group a09) and in the B-genome (linkage group b09) control three major fatty acids (oleic, linoleic and palmitic acid) [3,20,24,25]. The linked allele-specific [26] and cleaved amplified polymorphic sequences (CAPS) [27] markers for both the *ahFAD2* genes (*ahFAD2A* and *ahFAD2B*) are available for use in molecular breeding. Therefore, we deployed here two molecular breeding approaches namely, MABC and marker-assisted selection (MAS) to transfer the mutant alleles (*ahFAD2A* and *ahFAD2B*) in three elite genotypes. Keeping in mind the diverse industry preferences, two types of selection criteria were imposed (a) breeding lines with high oil and oleic acid content (Selection Criteria I), and (b) breeding lines with low oil content and high oleic acid (Selection Criteria II).

2. Materials and methods

2.1. Plant material

Three peanut genotypes namely, ICGV 06110, ICGV 06142 and ICGV 06420 were selected for improving oil quality. The genotypes were selected based on six-seasons of evaluation conducted during 2008–2010/11 rainy and post-rainy seasons at ICRISAT-Patancheru, India. The oil content of ICGV 06110 varied from 41.3 to 46.7% over six seasons, ICGV 06142 recorded an oil content varying from 52.5 to 58.2% and ICGV 06420 recorded 52.4 to 60.0%. The ICGV 06110, a Virginia bunch cultivar with medium maturing duration was derived from the cross [{[V79 × [(ICGV 86031 × ICGV 86030) × JL 24]] × ICGV 88386} × ICG 12348]. The ICGV 06142, a Spanish bunch genotype has medium maturity duration, and was derived from the cross [(ICGV 92069 × ICGV 93184) × (ICGV 96246 × 92R/75)]. The ICGV 06420, a Virginia bunch cultivar is a drought tolerant genotype and was derived from the cross ICGV 87846 × ICGV 99240. The SunOleic 95R is a high oleic acid (~80%) line developed by Florida Experimental Agriculture Station, USA using F435 [20] and is a low oil containing (45%) genotype. This genotype carries mutation in both *ahFAD2A* and *ahFAD2B* genes and been used as donor for improving oil quality in the above mentioned three genotypes.

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Table 1
Details of associated markers used for selecting *ahFAD2A* and *ahFAD2B* mutant alleles in breeding populations.

Type of markers	Gene	Markers	Wild allele size (bp)	Mutant allele size (bp)	References
Allele specific polymerase chain reaction (AS-PCR)	*ahFAD2A*	F435-F and F435SUB-R	Null allele	203	Chen et al. [26]
	ahFAD2B	F435-F and F435INS-R	Null allele	195	Chen et al. [26]
Cleaved amplified polymorphic sequences (CAPS)	*ahFAD2A*	aF19F and 1056R	598 and 228	826	Chu et al. [21]
	ahFAD2B	bF19F and R1/FADR	736, 263 and 171	550, 263, 213 and 171	Chu et al. [21]

2.2. Molecular markers

Two types of markers linked to *ahFAD2* genes were used to screen the breeding population for both the mutant alleles. The allele specific-polymerase chain reaction (AS-PCR) markers [26] were used for confirming hybridity of F_1 plants and identifying heterozygous plants in BC_1F_1 generation. The cleaved amplified polymorphic sequences (CAPS) markers [27] were deployed to identify plants with homozygous alleles for both the mutant alleles. The details of the markers used for screening are provided in Table 1.

2.3. DNA extraction and marker genotyping

The plants were labelled and leaf samples from the 10–15 days old seedlings were collected. The DNA was extracted from the parental genotypes and different segregating breeding progenies that include F_1s, BC_1F_1s, F_2s and BC_1F_2s, using the modified cetyltrimethyl ammonium bromide (CTAB) extraction method, as described in Mace et al. [28]. After DNA isolation, the quality and quantity were checked on 0.8% agarose gels. Based on the quality check results, the DNA concentration was normalized to ~5 ng/μl and used for genotyping breeding progenies with linked markers for *ahFAD2A* and *ahFAD2B* genes (Table 1).

2.3.1. Genotyping with allele specific-polymerase chain reaction markers

The *ahFAD2A* and *ahFAD2B* genes are located at the linkage group a09 in A-genome and linkage group b09 in B-genome, respectively. Two different primer pairs were required for amplifying the mutant alleles of *ahFAD2A* and *ahFAD2B* genes along with two other primer pairs to amplify a common allele for wild type allele as a control [26]. The primer combination, F435-F and F435SUB-R, amplified 203 bp fragment for the mutant allele (substitution from G:C → A:T) in the A-genome, while the primer combination, F435-F and F435INS-R amplified 195 bp fragment for the mutant allele (A:T insertion) in the B-genome. The primer combination, F435-F and F435IC-R was used as internal control to confirm successful amplification by amplifying 250 bp fragment for wild type allele.

The polymerase chain reaction (PCR) for AS-PCR markers was performed using the DNA samples, linked markers and other chemical components for amplification of target loci. The PCR was setup in 10 μl volume using 5 ng of genomic DNA together with 5 picomole primer (forward and reverse each), 1X PCR buffer (Sib-Enzyme, Russia), 5 mM $MgCl_2$, 0.03 U/μl of *Taq* DNA polymerase (Kapa Biosystems Inc, USA), and 0.2 mM dNTPs. The amplification was done in ABI thermal cycler (Applied Biosystems, USA). The PCR program included initial denaturation step for 3 min at 94 °C, first 5 cycles at 94 °C for 20 s, 65 °C for 20 s and 72 °C for 30 s, with 1 °C decrease in temperature each cycle. The remaining 40 cycles were performed at 94 °C for 20 s with constant annealing temperature (59 °C) for 20 s. The condition for primer extension was set at 72 °C for 30 s and final extension at 72 °C for 20 min. The amplified PCR products were then separated by electrophoresis at 150 V for 1 h on a 3.0% agarose gel (SeaKem LE Agarose, USA) in 1X TBE buffer. The ethidium bromide was used for staining the fragments and UV light was used for visualizing the stained fragments for easy scoring. While running the agarose gel, 100 bp DNA ladder (Life technologies, USA) was used as size reference for amplified fragments.

2.3.2. Genotyping with CAPS markers

The PCR amplification was performed in ABI thermal cycler (Applied Biosystems, USA). The primers aF19F and 1056R with a single *Hpy* 99I recognition site were used to detect the 448 G > A mutation in the *ahFAD2A* allele. Similarly, the primers bF19F and R1/FADR were used to detect 441_442ins A mutation in the *ahFAD2B* allele. The PCR mix consisted of 2–5 ng of DNA, 5 picomole each of forward (F) and reverse (R) primers, 2 mM $MgCl_2$, 2 mM dNTPs, 0.1 U of Taq DNA polymerase (Kapa Biosystems Inc, USA) and 1X PCR buffer. A standardized PCR program was used with 5 min of initial denaturation, followed by 32 cycles of 94 °C for 20 s, 52 °C for 40 s and 72 °C for 40 s ending with extension for 15 min at 72 °C [27]. The PCR product was resolved on 1.5% agarose gel for confirming the amplification and digested with restriction enzyme after purification.

For A-genome, the 0.5 U of restriction enzyme *Hpy99I* (New England Biolabs, UK) was used in restriction digestion of the 10 μl of A-genome amplicon by incubating at 37 °C for about 4 h. In case of wild type *ahFAD2A* allele, the 826 bp fragment was digested to 598 bp and 228 bp while the mutant genotypes had the 826 bp fragment intact. In the case of B-genome, 2.0 U of restriction enzyme *Hpy188I* (New England Biolabs, UK) was used for digestion of 10 μl of PCR amplicon for about 16 h at 37 °C. The wild type *ahFAD2B* allele of 1214 bp with five restriction sites cleaved into five fragments i.e., 736, 263, 171, 32 and 12 bp. The mutant allele has one additional restriction site (six restriction sites) in the 736 bp fragment which was further cleaved into 550 and 213 bp.

2.4. Hybridization and generation advancement

The SunOleic 95R was used as male donor parent, while ICGV 06110, ICGV 06142 and ICGV 06420 as female parents (Fig. 1). Well-developed buds from female parent were selected for emasculation, and anthers were carefully removed from the selected flower. Pollination was done next morning, by plucking a flower from male parent and squeezing pollen gently on the stigma of emasculated flower [29]. The F_1s were grown in next season and were genotyped with linked allele specific markers to identify true F_1 plants (Fig. 2A). From here onwards, two molecular breeding programs were followed. The MABC approach was deployed in all the three crosses, while the MAS approach was deployed for the one cross with the parent, ICGV 06420.

2.4.1. Generation of MABC introgression lines

The true F_1 plants were used as pollen donor and recurrent parents as female parent for making 1st backcross (Fig. 1). Three crosses namely ICGV 06110 × SunOleic 95R, ICGV 06142 × SunOleic 95R and ICGV 06420 × SunOleic 95R were designated as MABC Cross-I, MABC Cross-II and MABC Cross-III, respectively. The BC_1F_1 pods were harvested and seeds were planted in next season to grow BC_1F_1 plants. These plants were then genotyped with linked allele specific markers to identify heterozygous plants at both the loci. The BC_1F_2 pods obtained from selfing of the selected BC_1F_1 plants were harvested. The harvested BC_1F_2 seeds were planted

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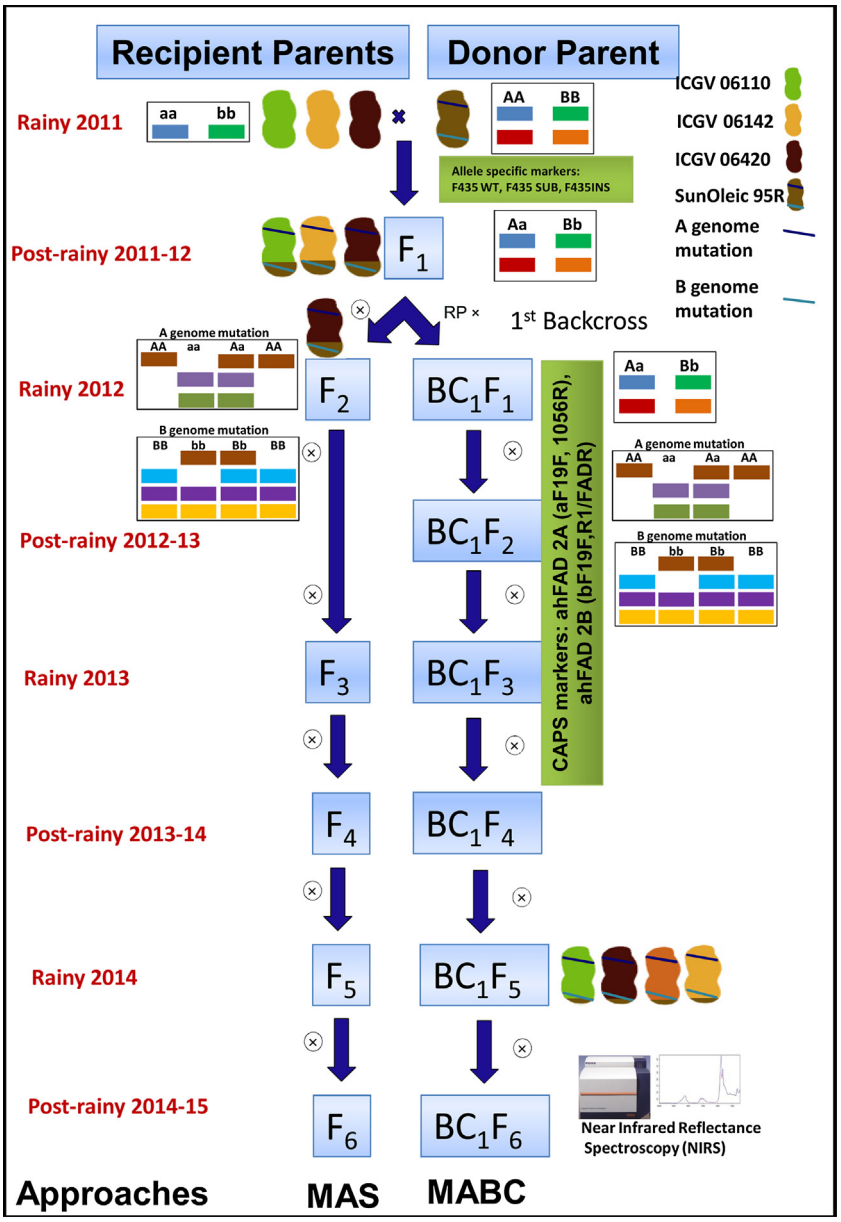

Fig. 1. Schematic diagram of molecular breeding approaches to develop MABC and MAS lines with improved oil quality keeping intact the oil content in three peanut cultivars. This illustration shows the development of MABC lines from three crosses while development of MAS lines for one cross. The allele-specific and CAPS markers linked to A- and B-genome mutant alleles were deployed for tracking them in the breeding populations over generations.

in next season and plants with both the homozygous mutant loci were identified using CAPS markers (Fig. 2B and C). These plants were selfed and $BC_1F_{2:3}$ pods were harvested. All the selected plants in BC_1F_3 were advanced to BC_1F_4 generation. Progeny rows of BC_1F_4 generation were planted in the field in single row and best plants/progenies based on morphological observations such as plant phenotype, number, size and shape of pods and maturity duration were selected for generation advancement. Similar selection was exercised in BC_1F_5 progeny rows to allow more homozygosity to come in the breeding population. Finally the seeds harvested from selected BC_1F_5 progenies were used to phenotype for oil and fatty acid composition using near infra-red reflectance spectroscopy (NIRS).

2.4.2. Generation of MAS introgression lines

In this approach, plants selected after genotyping F_1 population from the cross ICGV 06420 × SunOleic 95R (MAS Cross) were selfed (Fig. 1) to obtain F_2 generation pods. The F_2 seeds were planted and genotyped with CAPS markers. The plants with homozygous loci at both the mutated loci were identified and selfed. The generation advancements were done from F_2 to F_5 by single plant progeny rows followed by selecting superior lines based on morphological features upto F_5 generations. The F_6 seeds harvested from selected F_5 progeny rows were phenotyped for oil and fatty acid composition.

2.5. Field evaluation

The progenies were raised in experimental plots of Alfisols (Alfisol-Patancheru Soil Series; UdicRhodustolf) at ICRISAT, Patancheru, India (at 17.53° N latitude and 78.27° E, 545 m.a.s.l.) during 2014 rainy season. The selected progenies in F_5 and BC_1F_5 generations were raised in 2 m rows with spacing of 30 cm between rows, and 10 cm from plant to plant in a row. Recommended

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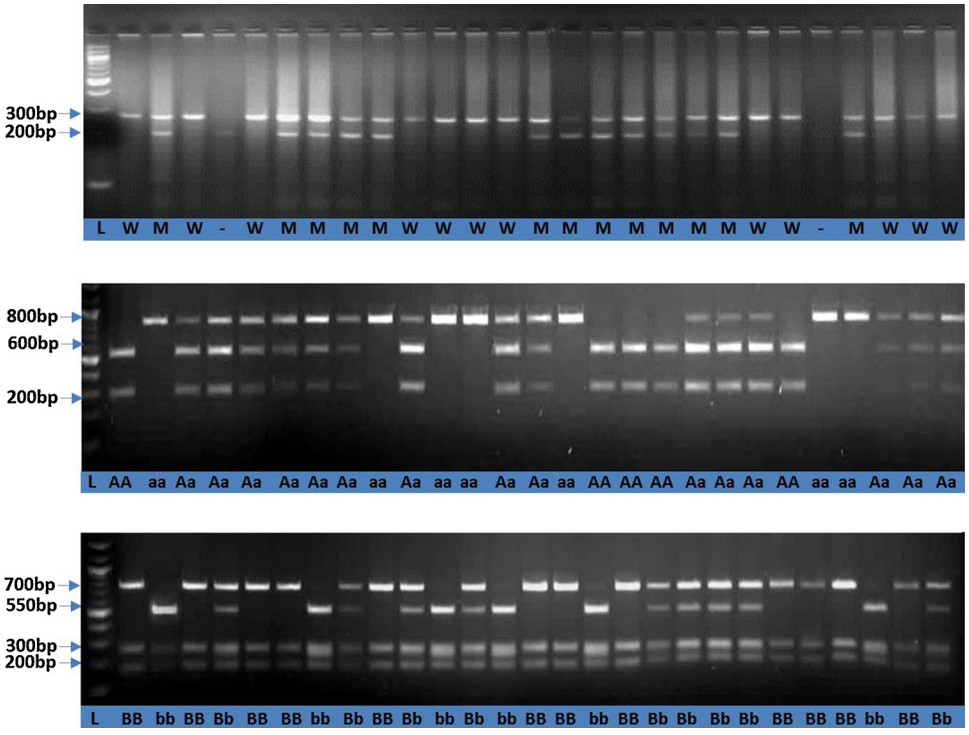

Fig. 2. Marker-assisted selection of breeding lines for both the mutant alleles. The figure (A) showed genotyping of BC_1F_1 generation with allele-specific markers for selection of heterozygous alleles for B-genome mutation. The 'L', 'M' and 'W' indicate DNA ladder, mutant allele and wild allele, respectively. The figure (B) showed genotyping of BC_1F_2 generation with CAPS marker for differentiating heterozygous and homozygous alleles for A-genome mutation. The 'L', indicates DNA ladder, 'AA' indicates homozygous wild allele of recurrent parent, 'Aa' indicates heterozygosity while 'aa' indicates homozygous mutant allele from donor parent. The figure (C) showed genotyping of BC_1F_2 generation with CAPS marker for differentiating heterozygous and homozygous alleles for B-genome mutation. The 'L', indicates DNA ladder, 'BB' indicates homozygous wild allele of recurrent parent, 'Bb' indicates heterozygosity while 'bb' indicates homozygous mutant allele from donor parent.

agronomic management practices were adopted to raise a healthy crop. Fertilizers included, basal application of 60 kg phosphorus pent-oxide (P_2O_5), and gypsum @ 400 kg/ha at peak flowering stage. Seed treatment was done with mancozeb @ 2 g/kg seed, and imidachloprid @ 2 ml/kg seed. To manage weeds, pendimethalin @ 1 kg active ingredient/ha was used as pre-emergence application to the soil. The experimental plot was protected against insect pests and foliar fungal diseases by spraying insecticides and fungicides at appropriate stages when crossed above threshold levels of damage. All the plants in each progeny row were harvested at maturity and dried in the field for 2 days before stripping the pods from plants. The stripped pods were cleaned for soil particles and dried further under shade to bring down the moisture content. Fully matured pods from each sample were shelled to obtain kernels for biochemical analysis.

2.6. Biochemical analysis for oil content and quality traits

The oil content and fatty acid composition were estimated using NIRS (model XDS RCA, FOSS Analytical AB, Sweden, Denmark) [30,31]. Earlier to this analysis, calibrations for estimation of fatty acids were done using gas chromatography (GC) estimates of 264 peanut samples (unpublished data). Similarly, calibration for oil content was done using Soxhlet estimates on 142 genotypes with oil content ranging from 40% to 57%. The conversion of fatty acids to methyl esters was carried out according to the protocol of Metcalf et al. [32]. Oil content estimation using Soxhlet method was also done following the protocol of Sharma et al. [33]. The regression coefficient (R^2) value for predicting oleic and linoleic acid using the calibration equation was 0.96, while 0.87 for palmitic acid and 0.83 for oil content. Non-destructive method of estimation was used in NIRS. Approximately 70–100 gm of each intact sample was scanned in a rectangular cup. Using these calibrations, the efficiency of cross validation, measured as coefficient of determination of cross validation (1−VR, where VR is variance ratio) was 0.94 for oleic and linoleic acid while 0.80 for palmitic acid and oil content.

3. Results

3.1. Development of MABC introgression lines

By using MABC approach 82 ILs_{MABC} were generated in the genetic background of three elite genotypes (ICGV 06110, ICGV 06142 and ICGV 06420). Details on the number of plants sampled in each generation for planting, marker screening and the number of positive plants are given in Supplementary Table 1. It is important to mention that the hybridizations were carried out in green house with a success rate of ~70%.

During 2011 rainy season, three crosses were made between recurrent and donor parents (MABC Cross-I, II and III) and harvested 103 F_1 seeds. During the next season i.e., 2011–12 post-rainy, all F_1 seeds were planted and 93 of these plants were screened with allele-specific markers. A total of 55 F_1 plants were found "true hybrids" carrying the target mutated gene's alleles (Supplementary Table 1). All 55 F_1 plants i.e., 13 from MABC Cross-I, 31 plants from MABC Cross-II and 11 plants from MABC Cross-III were used as pollen parents to make the first backcross with the respective recurrent parents. From these crosses, 125 BC_1F_1 seeds i.e., 17 seeds from MABC Cross-I, 62 seeds from MABC Cross-II and 46 seeds from MABC Cross-III were harvested at the end of post-rainy 2011–12.

In the next season (rainy 2012), all 125 BC_1F_1 seeds were planted and genotyped with allele-specific markers to select plants with mutant alleles for *ahFAD2A* and *ahFAD2B* genes in heterozygous condition (Fig. 2A). As a result, 21 BC_1F_1 plants (4 from MABC

Table 2
Best promising MABC introgression lines selected following the *Selection Criteria I* (high oil content, high oleic acid, low linoleic acid and low palmitic acid).

S. No.	MABC line	Oil content (%)	% change in oil content[d]	Oleic acid (%)	% increase in oleic acid[d]	Linoleic acid (%)	% decrease in linoleic acid[d]	Palmitic acid (%)	% decrease in palmitic acid[d]
SunOleic 95R (donor parent)		45.0		78.3		6.5		6.0	
ICGV 06110 (recurrent parent)		43.5		46.7		32.3		11.0	
1	IL_{MABC}HOG573[a]	56.9	30.8	72.2	54.6	11.5	64.4	7.4	32.7
2	IL_{MABC}HOG575[a]	54.4	25.1	70.0	49.9	12.0	62.8	8.2	25.5
3	IL_{MABC}HOG576[a]	54.5	25.3	67.0	43.5	16.3	49.5	7.7	30.0
ICGV 06142 (recurrent parent)		53.4		42.5		37.6		10.7	
4	IL_{MABC}HOG767[b]	55.5	3.9	79.4	86.8	4.6	87.8	6.4	40.2
5	IL_{MABC}HOG766[b]	55.3	3.6	81.3	91.3	3.9	89.6	5.9	44.9
6	IL_{MABC}HOG759[b]	54.6	2.2	77.5	82.4	6.3	83.2	6.9	35.5
7	IL_{MABC}HOG760[b]	54.1	1.3	78.7	85.2	5.0	86.7	7.4	30.8
ICGV 06420 (recurrent parent)		52.9		38.3		40.2		12.4	
8	IL_{MABC}HOG591[c]	57.9	9.5	78.4	104.7	6.1	84.8	6.5	47.6
9	IL_{MABC}HOG632[c]	56.4	6.6	67.3	75.7	15.2	62.2	7.7	37.9
10	IL_{MABC}HOG603[c]	55.1	4.2	71.8	87.5	12.4	69.2	7.7	37.9
11	IL_{MABC}HOG590[c]	54.8	3.6	72.1	88.3	13.2	67.2	7.2	41.9
12	IL_{MABC}HOG619[c]	54.6	3.2	71.4	86.4	13.5	66.4	6.9	44.4
13	IL_{MABC}HOG598[c]	54.5	3.0	69.8	82.2	13.2	67.2	8.3	33.1
14	IL_{MABC}HOG593[c]	54.0	2.1	71.8	87.5	12.7	68.4	7.1	42.7
15	IL_{MABC}HOG604[c]	54.0	2.1	74.4	94.3	10.2	74.6	6.5	47.6
16	IL_{MABC}HOG611[c]	53.9	1.9	67.2	75.5	15.0	62.7	8.7	29.8
17	IL_{MABC}HOG735[c]	53.6	1.3	73.3	91.4	10.9	72.9	7.2	41.9

[a] Pedigree: ICGV 06110 × (ICGV 06110 × SunOleic 95R).
[b] Pedigree: ICGV 06142 × (ICGV 06142 × SunOleic 95R).
[c] Pedigree: ICGV 06420 × (ICGV 06420 × SunOleic 95R).
[d] with respect to recurrent parent.

Cross-I, 10 plants from MABC Cross-II and 7 plants from MABC Cross-III) were found heterozygous. These plants were selfed and 292 BC_1F_2 seeds were harvested. All 292 BC_1F_2 seeds were planted during post-rainy 2012–13 and were genotyped with CAPS markers to select plants with mutant alleles for *ahFAD2A* and *ahFAD2B* in homozygous condition (Fig. 2B and C). As a result, 25 BC_1F_2 plants (1 from MABC Cross-I, 7 plants from MABC Cross-II and 17 plants from MABC Cross-III) were found homozygous. These plants were subsequently selfed and $BC_1F_{2:3}$ seeds were harvested separately from individual plants.

All $BC_1F_{2:3}$ seeds harvested from 25 BC_1F_2 plants were planted in a row (total 25 rows) during the rainy 2013 and made 183 single plant selections (15 from MABC Cross-I, 54 plants from MABC Cross-II and 114 plants from MABC Cross-III). The seeds were harvested from all the single plant selections at the end of season rainy 2013. In the next season i.e., post-rainy 2013–14, all the BC_1F_4 seeds were planted as a single plant progenies and made 82 single plant selections (6 from MABC Cross-I, 15 from MABC Cross-II and 61 from MABC Cross-III) and lines were referred as ILs_{MABC}. The seeds were harvested, bulked from these 82 BC_1F_5 plants (ILs_{MABC}) and used for estimation of oil content and quality traits.

3.2. Development of MAS introgression lines

The MAS approach was deployed for the cross ICGV 06420 × SunOleic 95R (MAS Cross) (Fig. 1). A total of 32 F_1s produced from the MAS cross during the rainy 2011 were planted during the post-rainy 2011–12 and screened with allele-specific markers (Supplementary Table 1). Marker analysis of these F_1s resulted in identification of 11 "true hybrids" carrying the mutated alleles of the genes in heterozygous condition (Fig. 2A). These 11 F_1 plants produced a total of 462 F_2 seeds. During the rainy 2012, all the 462 F_2 seeds were planted and genotyped with CAPS markers to select plants with mutant alleles for *ahFAD2A* and *ahFAD2B* in homozygous condition (Fig. 2B and C). Marker analysis resulted in identification of 17 plants carrying the mutated alleles for both the *ahFAD2* genes in homozygous condition. Total selfed seeds from these plants were harvested individually. The harvested seeds ($F_{2:3}$) from each of these 17 plants were planted in rows (17 rows) during rainy 2013 season. A total of 112 single plant selections were made and seeds were harvested from these selected plants. During post-rainy 2013–14, the 112 F_4 plants were sown in rows (2 m) and selfed seeds from 210 single plant selections were harvested. All the harvested F_5 seeds were sown in single plant progeny rows during rainy 2014 and selections were made based on yield and pod features. Finally, a total of 387 single plant/bulk selections were made and referred as ILs_{MAS}. All these ILs_{MAS} were then used for estimation of oil content and oil quality. After the phenotyping, the *Selection Criteria I* and *Selection Criteria II* were practiced and best lines were identified for evaluation trials during post-rainy 2014–15 season.

3.3. Biochemical analysis of MABC and MAS ILs for oil content and quality traits

All 82 ILs developed through MABC and 387 ILs derived through MAS approach were evaluated for oil content, oleic acid, linoleic acid and palmitic acid. In the genetic background of ICGV 06110 (MABC Cross-I), 6 ILs_{MABC} (BC_1F_6 generation) were phenotyped for oil content and three major fatty acids. The oil content among these lines ranged from 49.33% to 56.90% and recorded an average of 53.0% (Supplementary Table 2). The oleic acid ranged from 62.09% to 72.16% with an average of 67.0% while the linoleic acid ranged from 11.54% to 20.54% with an average of 15.82%. We observed 33–55% increase in oleic acid content among these ILs as compared to the recurrent parent, ICGV 06110. On the other hand, the linoleic acid levels showed 36–64% decrease as compared to the recurrent parent, ICGV 06110. The palmitic acid varied from 7.39% to 9.0% with an average of 8.14%, which is a reduction of 18–33% compared to the recurrent parent ICGV 06110. Of these 6 lines, 3 ILs_{MABC} (IL_{MABC}HOG573, IL_{MABC}HOG575 and IL_{MABC}HOG576) were selected following the *Selection Criteria I* (Table 2) while one ILs_{MABC} (IL_{MABC}HOG574) following the *Selection Criteria II* (Table 3).

Similarly in the genetic background of ICGV 06142 (MABC Cross-II), 15 ILs_{MABC} (BC_1F_6 generation) were subjected to biochemical analysis. The oil content among these lines ranged from 46% to 56% (Supplementary Table 2). The oleic acid ranged from 62.30% to 81.30% with an average of 72.5%. In comparison to ICGV 06142,

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Table 3
Best promising MABC introgression lines selected following the *Selection Criteria II* (low oil content, high oleic acid, low linoleic acid and low palmitic acid).

S. No.	MABC line	Oil content (%)	% change in oil content[d]	Oleic acid (%)	% increase in oleic acid[d]	Linoleic acid (%)	% decrease in linoleic acid[d]	Palmitic acid (%)	% decrease in palmitic acid[d]
SunOleic 95R (donor parent)		45.0		78.3		6.0		6.5	
ICGV 06110 (recurrent parent)		43.5		46.7		32.3		11.0	
1	IL_{MABC}HOG574[a]	49.3	13.3	64.5	38.1	18.0	44.3	8.6	21.8
ICGV 06142 (recurrent parent)		53.4		42.5		37.6		10.7	
2	IL_{MABC}HOG764[b]	46.4	−13.1	69.5	63.5	12.4	67.0	7.8	27.1
3	IL_{MABC}HOG765[b]	47.1	−11.8	68.3	60.7	13.5	64.1	7.8	27.1
4	IL_{MABC}HOG770[b]	47.3	−11.4	62.3	46.6	17.7	52.9	9.1	15.0
5	IL_{MABC}HOG763[b]	47.4	−11.2	64.8	52.5	16.5	56.1	8.2	23.4
6	IL_{MABC}HOG758[b]	47.7	−10.7	69.4	63.3	13.1	65.2	7.7	28.0
7	IL_{MABC}HOG762[b]	48.7	−8.8	69.0	62.4	12.6	66.5	8.2	23.4
8	IL_{MABC}HOG769[b]	49.5	−7.3	75.5	77.6	7.8	79.3	7.4	30.8
9	IL_{MABC}HOG757[b]	49.9	−6.6	75.3	77.2	7.7	79.5	7.0	34.6
ICGV 06420 (recurrent parent)		53.0		38.3		40.2		12.4	
10	IL_{MABC}HOG580[c]	48.5	−8.3	71.8	87.5	9.4	76.6	7.6	38.7
11	IL_{MABC}HOG579[c]	48.6	−8.1	67.0	74.9	16.6	58.7	7.8	37.1
12	IL_{MABC}HOG743[c]	49.0	−7.4	65.1	70.0	17.5	56.5	7.9	36.3
13	IL_{MABC}HOG584[c]	49.4	−6.6	68.5	78.9	14.1	64.9	7.9	36.3
14	IL_{MABC}HOG585[c]	49.4	−6.6	64.3	67.9	17.1	57.5	8.8	29.0
15	IL_{MABC}HOG682[c]	49.5	−6.4	70.5	84.1	12.4	69.2	7.5	39.5
16	IL_{MABC}HOG666[c]	49.6	−6.2	63.8	66.6	18.5	54.0	8.6	30.6
17	IL_{MABC}HOG610[c]	49.9	−5.7	69.7	82.0	11.2	72.1	8.2	33.9
18	IL_{MABC}HOG609[c]	49.9	−5.7	68.0	77.5	11.7	70.9	8.8	29.0

[a] Pedigree: ICGV 06110 × (ICGV 06110 × SunOleic 95R).
[b] Pedigree: ICGV 06142 × (ICGV 06142 × SunOleic 95R).
[c] Pedigree: ICGV 06420 × (ICGV 06420 × SunOleic 95R).
[d] with respect to recurrent parent.

47–91% increase in oleic acid levels was found among the ILs. The linoleic acid ranged from 3.9% to 17.73% with an average of 10.4% while the palmitic acid ranged from 5.93% to 9.06% with an average of 7.46%. In contrast to oleic acid, we observed 53–90% decrease in linoleic acid and 15–45% decrease in palmitic acid among ILs as compared to the recurrent parent ICGV 06142. Of these 15 lines, 4 ILs_{MABC} (IL_{MABC}HOG759, IL_{MABC}HOG760, IL_{MABC}HOG766 and IL_{MABC}HOG767) with high oil content qualified under *Selection Criteria I* (Table 2) and 8 ILs with low oil content qualified under *Selection Criteria II* (Table 3).

Similarly in the genetic background of ICGV 06420 (MABC Cross III), 61 MABC ILs (BC_1F_6 generation) were subjected to biochemical analysis. The oil content among these lines ranged from 48.45% to 57.93% (Supplementary Table 2). The oleic acid ranged from 62.27% to 78.4% with an average of 68.9%. In terms of increase in percentage among ILs, 63–105% increase was observed in oleic acid levels as compared to recurrent parent, ICGV 06420. The linoleic acid ranged from 6.10% to 19.88% with an average of 14.1% while the palmitic acid ranged from 6.10% to 9.01% with an average of 7.90%. In contrast to oleic acid, 51–85% decrease in linoleic acid and 27–51% decrease in palmitic acid was observed among ILs as compared to the recurrent parent ICGV 06420. Of these 61 lines, 10 ILs with high oil content were selected following *Selection Criteria I* (Table 2, Fig. 3A) while 9 ILs with low oil content following *Selection Criteria II* (Table 3).

Similar to ILs_{MABC}, all 387 ILs_{MAS} from the cross ICGV 06420 × SunOleic 95R (MAS Cross) were analyzed for estimation of oil content and oil quality traits (oleic acid, linoleic acid and palmitic acid). The oil content among these lines ranged from 42.0% to 55.1% (Supplementary Table 3). The oleic acid ranged from 65.1% to 82.6% with an average of 71.5% while the linoleic acid ranged from 2.1% to 16.8% with an average of 10.3%. We observed 70–116% increase in oleic acid among these ILs as compared to the parent ICGV 06420. On the other hand, we observed 58–95% decrease in linoleic acid as compared to the parent ICGV 06420. The palmitic acid ranged from 5.7% to 9.5% with an average of 7.9%. Similar to linoleic acid, a reduction of 23–54% was seen in palmitic acid as compared to the parent ICGV 06420. The above mentioned variation among the ILs_{MAS} allowed us to select the 10 best and promising lines with best combination of fatty acid profile each following the *Selection Criteria I* (Table 4, Fig. 3B) and *II* (Table 4, Fig. 3C).

4. Discussion

High oleic peanuts either as kernels or oils are preferred by both consumers and food processing industries due to their multiple health benefits and enhanced shelf life. Thus breeding of high oleic peanut lines is an important aspect of peanut improvement programs worldwide. The enzyme, fatty acid desaturase (*ahFAD2*) catalyzes the conversion of oleic to linoleic acid, and is encoded by two homeologous genes, *ahFAD2A* and *ahFAD2B*, located on the A- and B-genome, respectively [34–36]. Both the *ahFAD2* genes have 99% sequence homology and inactivation of both the genes is required for accumulation of high oleic acid in plants. The identification of molecular markers linked to both *ahFAD2* genes in peanut has made it possible to target this trait in peanut improvement programs, a process referred to as marker-assisted breeding. The MABC ensures that only the target gene/QTL is transferred while keeping the other features of the original recurrent parent intact [22,23]. Using this approach, nematode resistance [37], high oleic acid [38] and rust resistance [39] were earlier transferred to cultivated and popular peanut lines. MAS approach on the other hand involves selection of plants/progenies in segregating generation derived from a cross using molecular markers. Unlike in MABC, recovery of recurrent parent is not the objective in MAS.

Both, conventional and molecular breeding approaches were used to transfer the high oleate trait into popular peanut genotypes. The F435, a mutant peanut line with 80% oleic acid and 2% linoleic acid was the initial source for high oleate trait [19]. In the absence of linked markers, conventional breeding methods were used to breed high oleate peanut lines such as, SunOleic 95R [20], Tamrun OL01 [40] etc. The first instance of using MABC targeting the high oleate trait in peanut was reported by Chu et al. [38] who also developed the markers for *ahFAD2A* [21] and *ahFAD2B* genes [27] in peanut. The CAPS markers were used to monitor transfer of high oleic acid trait into the nematode resistant genotype 'Tifguard'

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Table 4
Best promising MAS introgression lines following the *Selection Criteria I* and *II*.

S. No.	MAS line	Oil content (%)	% change in oil content[a]	Oleic acid (%)	% increase in oleic acid[a]	Linoleic acid (%)	% decrease in linoleic acid[a]	Palmitic acid (%)	% decrease in palmitic acid[a]
SunOleic 95R (donor parent)		45.0		78.3		6.0		6.5	
ICGV 06420 (recurrent parent)		53.0		38.3		40.2		12.4	
Selection Criteria I (high oil content, high oleic acid, low linoleic acid and low palmitic acid)									
1	IL_{MAS}HOG373	55.1	4.2	74.9	95.6	9.4	76.6	7.2	41.9
2	IL_{MAS}HOG232	54.7	3.4	81.9	113.8	2.5	93.8	6.0	51.6
3	IL_{MAS}HOG334	54.7	3.4	79.4	107.3	5.0	87.6	6.7	46.0
4	IL_{MAS}HOG354	54.5	3.0	74.5	94.5	7.9	80.3	7.7	37.9
5	IL_{MAS}HOG220	54.2	2.5	82.6	115.7	2.6	93.5	6.2	50.0
6	IL_{MAS}HOG407	54.0	2.1	72.8	90.1	9.6	76.1	8.2	33.9
7	IL_{MAS}HOG470	54.0	2.1	74.5	94.5	8.5	78.9	7.7	37.9
8	IL_{MAS}HOG208	53.6	1.3	78.2	104.2	5.6	86.1	6.5	47.6
9	IL_{MAS}HOG562	53.5	1.1	79.4	107.3	4.4	89.1	6.6	46.8
10	IL_{MAS}HOG526	53.0	0.2	73.3	91.4	10.3	74.4	7.2	41.9
Selection Criteria II (low oil content, high oleic acid, low linoleic acid and low palmitic acid)									
1	IL_{MAS}HOG237	42.4	−19.8	71.8	87.5	9.6	76.1	7.9	36.3
2	IL_{MAS}HOG225	42.7	−19.3	71.7	87.2	8.5	78.9	7.8	37.1
3	IL_{MAS}HOG183	42.7	−19.3	72.5	89.3	8.1	79.9	8.1	34.7
4	IL_{MAS}HOG174	43.0	−18.7	73.9	93.0	7.3	81.8	7.6	38.7
5	IL_{MAS}HOG254	43.5	−17.8	72.5	89.3	8.1	79.9	7.8	37.1
6	IL_{MAS}HOG110	43.9	−17.0	71.3	86.2	11.1	72.4	8.1	34.7
7	IL_{MAS}HOG230	44.7	−15.5	74.7	95.0	6.0	85.1	7.3	41.1
8	IL_{MAS}HOG161	44.9	−15.1	77.4	102.1	2.2	94.5	7.2	41.9
9	IL_{MAS}HOG134	44.9	−15.1	77.6	102.6	2.1	94.8	7.4	40.3
10	IL_{MAS}HOG472	45.0	−14.9	72.4	89.0	9.5	76.4	7.4	40.3

[a] with respect to recurrent parent.

and the improved breeding line 'Tifguard High O/L' was developed with high oleic acid content. The CAPS and SNP markers linked to high oleic acid content were used to track the mutant alleles in the background of backcross breeding lines over the generations. This approach considerably reduced the time and the amount of breeding material in different backcross generations. Further confirmation of selected lines was also carried out through HybProbe SNP assay [41]. In another study, Mienie and Pretorius [42] used multiplex real-time PCR assay developed by Barkley et al. [43] for selecting the heterozygous and homozygous breeding lines for both the mutant alleles. The above studies indicated that MABC and MAS approach could be conveniently used to monitor the progeny performance in early generations of breeding program for high oleic acid content.

In our experiment, three elite peanut genotypes namely ICGV 06110, ICGV 06142 and ICGV 06420 were improved for oil quality using two molecular breeding approaches. Both, genotyping in early generations and phenotyping in advance generations were employed to select the lines with high oleic acid content and desirable oil content. Genotyping-based selection was done in early generations, to confirm hybridity in F_1, BC_1F_1, and discard a large number of unwanted plants, not confirming to presence of mutant *ahFAD2* alleles in F_2 and BC_1F_2 generations. Subsequently, selected progenies were phenotyped in advance generations i.e., F_5, F_6, BC_1F_5, and BC_1F_6 seeds, to select promising ILs for further evaluation. Among the selected marker homozygotes, greater proportion of ILs from MAS showed higher levels of oleic acid content compared to the proportion derived from MABC. A total of 82 MABC ILs that include 6 from MABC Cross-I, 15 from MABC Cross-II and 61 from MABC Cross-III, and 387 MAS ILs from MAS Cross were selected fulfilling both the selection criteria. Two selection criteria namely, *Selection Criteria I* that combines high oil content with high oleic acid and low linoleic and palmitic acid, and *Selection Criterial II* that combines low oil content with high oleic acid and low linoleic and palmitic acid were used. Several of the selected lines, are expected to meet the food processing industry need of high oleic acid of above 70%.

Selection Criteria I considered high oil and oleic acid content to identify lines suitable for oil industry to produce high quality oil. Besides, calorific value contributed by fat content is high in the product made from high oil containing peanuts, and is desirable in preparation of ready-to-use therapeutic food (RUTF) products used to treat acute malnutrition. Oil with high oleic acid content at least has two benefits, health benefits to consumer and enhanced shelf life. Studies have shown that high oil yielding peanuts have economic benefits to stakeholders of peanut oil value chain. Based on *Selection Criteria I*, 17 ILs_{MABC} and 10 ILs_{MAS} were selected. ILs_{MABC} includes, 3 lines from MABC Cross-I, 4 lines from MABC Cross-II and 10 lines from MABC Cross-III. The oil content in these lines was high and varied from 53.0 to 57.9%. Based on *Selection Criteria II*, 18 ILs from MABC crosses, which included one line from MABC Cross-I, 8 lines from MABC Cross-II and 9 lines from MABC Cross-III, and 10 ILs from MAS Cross were selected. The oil content in these lines varied from 42.4 to 49.9%. Low oil content peanuts are needed for table purposes, confections and several other food uses. The oleic acid content in donor parent, Sunoleic 95R, was 78.0% and among the selected ILs with high/low oil content, it varied from 62 to 83%. Besides, all the ILs, selected under both these criteria, recorded a decrease in linoleic acid by 0.4–1.0 folds, and palmitic acid by 0.1–0.6 as compared to recurrent parents. Reduced linoleic and palmitic acid contents have additional health benefits to consumers.

Interestingly, combination of high oleic acid with high oil containing ILs were derived from low oil containing recurrent parent, ICGV 06110. Similarly, combination of high oleic acid with low oil content among ILs were derived from high oil containing parents, ICGV 06142 and ICGV 06420. Despite the selected ILs being homozygotes for mutant *ahFAD2* alleles, their oleic acid content varied from 62 to 83%. These observations on oil content and oleic acid content are expected as a consequence of quantitative nature of these traits [44]. Furthermore, this may in part be attributed to contribution of alleles governing high oil content coming from a low oil line, indicating involvement of several QTLs and/or modifying genes determining oil content in peanut. Influence arising from

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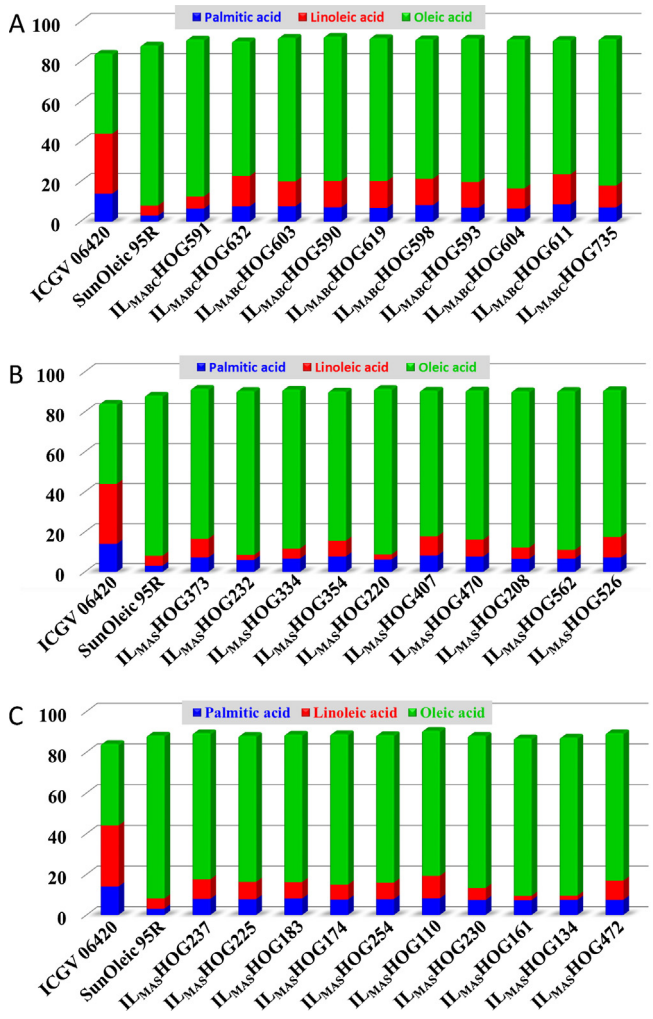

Fig. 3. MAS and MABC lines with increased oleic acid and desirable range of linoleic acid and palmitic acid in the genetic background of ICGV 06420. The figure showed (A) MABC lines with combination of high oil content and oleic acid, and low linoleic and palmitic acid; (B) MAS lines with combination of high oil content, high oleic acid, and low linoleic and palmitic acid, and (C) MAS lines with combination of low oil content and oleic acid, and low linoleic and palmitic acid.

new combination of alleles in the recurrent parent background and the interaction of these alleles with the environment could also be the reason for occurrence of new variants in the IL population. The probability of new variants was higher in MAS derived lines, where ILs with oil content as low as 42% were identified, as a consequence of significant contribution from recurrent parent. The selected lines based on their agronomic performance and stability in the yield evaluation trials will be useful to peanut oil extraction and food processing industry.

The combined selection approach of both genotypic-based as well as phenotypic-based selection was found suitable and effective in selecting improved lines with target traits, desired plant features, and agronomic value. Phenotypic selection was quite useful to identify the impact of the mutant alleles on the target trait. Significant variability for oleic acid content was found in the genotypes that were identified positive for the target allele, therefore, phenotypic confirmation was essential to advance the selected lines for further evaluations. Even in the case of selected ILs, the oleic acid content was found to vary from 62 to 82%. This huge variation in oleic acid content in spite of both the *ahFAD2* mutant alleles being in homozygous condition could be due to the effect of some modifying genes. Modifying genes are defined as genes whose only function is to intensify or diminish the expression of a major gene [45]. Such genes are very difficult to characterize due to their small effect on the trait of interest and very often by the masking effect of major genes [46]. Modifying genes were reported to be responsible for high oleic acid content variation in other oilseed plants like, safflower [47] and sunflower [48]. In safflower, the modifying gene was found to further increase oleic acid content in individuals homozygous for the *ol* allele [47].

The increase in oleic acid content of the ILs was followed by a concomitant reduction in the levels of linoleic acid. This was expected, since mutation in the *ahFAD2* allele resulted in inactivation of fatty acid desaturase enzyme which converted oleic acid to linoleic acid. However, considerable variation ranging from 2 to 20% was observed for linoleic acid content among the selected ILs. Different homeologes of the *ahFAD2* gene were reported in different crops, and their expressions were found to be strongly tissue specific. For example, in *Arabidopsis* and maize, a single copy of the *FAD2* gene was reported [49,50] while multiple copies of the gene were identified in the oil crops such as sunflower, soybean and canola [51–53]. In soybean, two different homeologes of *FAD2* were reported; *FAD2-1* was strongly expressed in developing seeds while *FAD2-2* was constitutively expressed in both vegetative tissue and developing seeds [54]. Besides genotype, linoleic acid content in developing seeds was also widely influenced by the environmental temperature [55,56]. High temperature during seed development could have decreased the linoleic acid content in the oil [57].

Among the progenies, which were confirmed with associated markers as homozygotes for both the *ahFAD2* mutant alleles, we observed higher number of progenies with high oleic acid content of ≥75% in MAS cross (69 plants), than that for MABC crosses (8 plants). This may be in part contributed by suppression effect of negative alleles in the recurrent parent or due to loss of minor effect positive alleles from donor parents. Single cross made between two parents in MAS, increased the chances of accumulating minor positive QTLs for the target trait, here oleic acid content, along with the major QTL from the donor parent is present in the background of the recipient parent. In contrary, the backcross program involved repeated crossing to recurrent parent and selection for major QTL and recovery recurrent parent genotype, and often ignored the effect of the minor QTLs. Hamdan et al. [47] reported that the loss of such minor effect QTLs or modifying genes can substantially alter the oleic acid content even on the higher side.

Studies concerning high oleic acid content have mostly focused on the levels of oleic acid and linoleic acid in the improved lines. Very often it was observed that the change in one metabolite brought about by a change in the corresponding enzyme in a biosynthetic pathway, affected the levels of all other metabolites in the pathway. Recent studies by Pandey et al. [22] and Wang et al. [58] showed that *ahFAD2* mutant alleles had also effect on palmitic acid levels. Even in our study, the ILs with mutant alleles showed significant reduction for palmitic acid. The most significant achievement of this study was that the original oil content (high oil content in case of ICGV 06142 and ICGV 06420 while low content in case of ICGV 06110) feature of the recurrent parent was successfully retained in the ILs. The improved lines with high/low oil content and improved oil quality upon further evaluation in national trials can be released as variety or used as parents in new breeding programs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.plantsci.2015.08.013.

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