BMC Plant Biology

RESEARCH Open Access

Marker-assisted introgression to improve the oleic acid content in the TMV 7 groundnut (*Arachis hypogaea* L.) variety suitable for the oil industry

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Abstract

Background Improving the quality and shelf life of groundnut oil is one of the foremost objectives of groundnut breeding programmes. This can be achieved by marker-assisted introgression, a technique that efficiently and precisely enables breeders to develop plants with enhanced qualities. This study focused on improving the oleic acid content of an elite groundnut variety, TMV 7, by introgressing a recessive mutation responsible for the increase in oleic acid from ICG 15419. Hybridization was performed between the donor and recurrent parents to develop the F_1, BC_1F_1, BC_2F_1 and BC_2F_2 populations. Introgressed lines with increased oleic acid in the genetic background of TMV 7 were identified using allele-specific marker, F435-F, F435SUB-R and a set of SSR markers were employed to recover the genome of the recurrent parent.

Results With two backcrosses, a total of ten homozygous plants in the BC_2F_2 population were identified with oleic acid content ranging from 54.23 to 57.72% causing an increase of 36% over the recurrent parent. Among the ten lines, the line IL-23 exhibited the highest level of recurrent parent genome recovery of 91.12%.

Conclusions The phenotypic evaluation of 10 homozygous introgressed lines indicated fewer differences for all other traits under study compared to the recurrent parent, except for oleic acid and linoleic acid content confirming the genetic background of the recurrent parent. The identified lines will be subjected to multilocation trials before their commercial release.

Keywords Quality trait, Backcross breeding, NIR estimation, Allele-specific primers, CAPS marker

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Background

Arachis hypogaea L., an annual herbaceous plant commonly known as groundnut or peanut belongs to the family of Fabaceae. It is considered a cash crop and plays a key role in global agriculture, food and economic development in many countries, making it an interesting and significant crop in different aspects of human life [1]. Groundnuts flourish in a warmer climate; therefore, the countries in the tropical and subtropical zones are the largest producers of this crop, with Brazil being the center of origin [2].

Groundnuts are excellent sources of quality and inexpensive protein with healthy fats minerals and bioactive compounds. They add to the utility in several ways, such as for oil production and its byproducts as animal feed; for confectionery and, additionally, as a legume crop, they contribute to sustainable agriculture by fixing nitrogen in the soil [3]. As one of the predominant sources of cooking oil, it is essential and mandatory to meet the requirements of agricultural research and development. In addition to quantity, the quality of the oil plays a pivotal role. Groundnut oil largely comprises unsaturated fatty acids (UFAs), which include oleic acid (C18:1), linoleic acid (18:2) and approximately 20% saturated fatty acids that include palmitic acid and stearic acid. Among the UFAs, polyunsaturated fatty acids are slightly more abundant than mono-unsaturated fatty acids (MUFAs) in elite cultivars [4]. Although the proportion of unsaturated fatty acids is greater in groundnut oil, it is largely prone to oxidation due to a lower oleic acid/linoleic acid (O/L) ratio [5], and the current varieties under cultivation have an O/L ratio of 1, and improving them even slightly would increase the storage life and health benefits of the oil. Furthermore, an increasing trend has been observed in the consumption of seed oils rich in omega-6 fatty acids, leading to an unbalanced consumption of omega-3 and omega-6 fatty acids, which might be harmful in the long run [6].

The genes responsible for the quality of groundnut oil are present on the ninth chromosome of both the A and B genomes. Two homologous genes, *ahFAD2A* and *ahFAD2B*, regulate the expression of the enzyme phosphatidyl-choline oleyl desaturase, which aids in the conversion of oleic acid to linoleic acid in normal groundnut genotypes. A natural groundnut mutant, F435 was discovered which contained 80% oleic acid and less than 10% linoleic acid because of the mutation of both genes, subsequently preventing the conversion of oleic acid to linoleic acid, leading to a greater quantity of the former [7]. The mutation in the A genome was due to the substitution of adenosine for guanosine at 448 bp, giving rise to a different amino acid, and the mutation in the B genome was an addition of adenosine at 442 bp, leading to a stop codon due to frame-shift mutation [8]. This identified line was further used in the development of a few high-oleic acid lines, such as SunOleic95R and SunOleic97R, which have since been involved in quality breeding [9]. An oleic acid content of up to 73% can be obtained with substitution, and such genotypes could also be used in groundnut breeding programmes [10].

Therefore, to fortify and extend the shelf life of groundnut oil, it is necessary to develop new varieties with improved oleic acid content, which can be achieved by backcross breeding programmes. In contrast to the conventional backcross breeding method, marker-assisted backcross (MABC) breeding is more precise because it involves the use of molecular markers. Markers closely linked to target genes can be obtained from genetic linkage maps and can be used to select introgressed lines at a very early stage and at a relatively high efficiency. Since the invention of markers, breeding new varieties pertaining to resistance, quality and other biotic and abiotic factors has the advantage of selecting only the desired gene from the donor parent by easily eliminating the rest of the donor genome. Additionally, as markers are not influenced by external factors, effective selection is possible. In recent decades, the MABC technique has been used to improve the oleic acid content in low-oleic superior varieties by crossing with an oleic-rich donor. [11–14].

In addition to being used in the food industry, oils with increased oleic acid content are used in the production of cosmetics, lubricants and biofuels due to their stable nature. The present study involved introgressing a recessive substitution mutation in the *ahFAD2A* allele from an oleic-rich donor into the background of an elite variety, TMV 7, via a marker-assisted backcross breeding program. The generations were advanced to BC_2F_2, and elite lines were selected by confirming their homozygous nature, oleic and linoleic acid estimations and recurrent parent genome recovery percentage.

Materials and methods

Experimental plant materials and work plan

An oleic-rich germplasm, ICG 15419, obtained from the International Crop Research Institute for Semi-Arid Tropics with oleic acid greater than 60% was used as the donor parent, and TMV 7, obtained from the Oilseed Research Station, Tindivanam, a selection from Tennessee with an oleic acid content of 40–44%, was used as the recurrent parent. Hybridization was carried out between the donor and recurrent parent to obtain F_1 plants. The F_1 plants positive for *ahFAD2A* mutation (Ol_1ol_1) were used as the pollen parent and were backcrossed with the recurrent parent to develop the BC_1F_1 population. The true hybrids in the BC_1F_1 population were again backcrossed with the recurrent parent to generate a second backcross population. The positive BC_2F_1 plants were selfed to produce the BC_2F_2 population (Fig. 1).

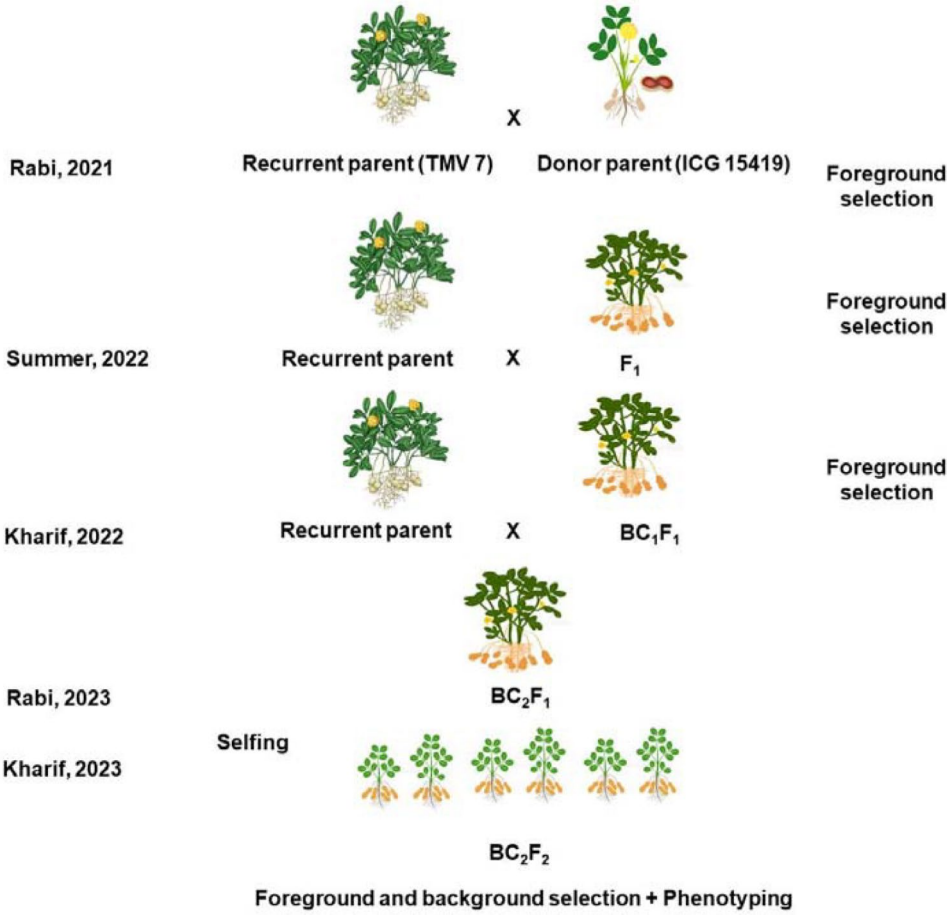

Fig. 1 Flowchart depicting the plan of the marker-assisted backcross breeding programme

Foreground selection

Genomic DNA was subsequently isolated from leaf samples of 14-day-old plants of the donor parent, recurrent parent, F_1, BC_1F_1, BC_2F_1 and BC_2F_2 populations [15]. DNA quantification was performed by using a Nano drop spectrophotometer (Thermoscientific, USA) and quality was checked by 0.8% agarose gel electrophoresis. The quantity of DNA was normalized to 40 ng/µl by diluting it with sterile distilled water. Allele-specific primers which included a forward primer F435-F and a reverse primer F435SUB-R along with an internal control, F435IC-R, were used to detect the substitution mutation in the A genome to ensure the success of the polymerase chain reaction [16]. The PCR cocktail mixture contained 2 µl of genomic DNA, 1 µl each of forward and reverse primers, 3 µl of master mix (smART Prime 2X) and 3 µl of nuclease-free water. The PCR program started with an initial denaturation of 4 min at 94 °C, followed by 35 cycles of denaturation for 30 s at 94 °C, annealing at 55 °C for 45 s and extension for one minute at 72 °C, with a final extension of 20 min at 72 °C. The PCR products were resolved on 3% agarose gels and visualized under UV light.

Confirmation of homozygosity by the cleaved amplified polymorphic sequence (CAPS) marker

A notable feature of the CAPS marker is the use of restriction digestion, where the enzyme cleaves the polymorphic region at specific recognition sites. The primers aF19 (forward) and 1056 (reverse) and the enzyme *Hpy99I* were used for the identification of homozygotes in the A genome [17]. To carry out the process of digestion, 3 µL of the amplified PCR product, 0.2 µL of the restriction enzyme, 1 µL of the restriction buffer and 6 µL of the nuclease-free sterile water were transferred to fresh PCR tubes and incubated at 37 °C for four hours. After digestion, 3 µL of the digested product was mixed with 2 µL of the loading dye, separated via agarose gel electrophoresis and visualized via UV.

Background selection

Simple sequence repeat (SSR) primers were used to detect polymorphisms between the donor and the recurrent parent. A total of 126 SSR primers extending across the 20 chromosomes were used in this study [18]. The percentage of parental polymorphism was estimated as suggested by [19].

Percentage of parental polymorphism = (number of polymorphic markers/total number of markers used) * 100.

Recurrent parental genome recovery (RPGR)

The molecular scoring data were used to calculate the recovery percentage of the recurrent parent genome and were analyzed using the software Graphical GenoType version 2.0. Visualizations from the software depicted the percentage of donor and recurrent parent in the selected progenies, and the recovery percentage was calculated using the following formula:

$$\mathrm{RPGR}\ \% = (\mathrm{R} + 0.5\mathrm{H}/\mathrm{P}) * 100$$

where R is the number of homozygous markers for the recurrent parent allele, H is the number of markers that were heterozygous and P is the total number of markers that showed polymorphism [19].

Phenotyping

Quality traits

The oil, oleic acid and linoleic acid contents were estimated using an NIR spectrophotometer (ZEUTEC, Germany). Approximately 8–10 groundnut kernels were powdered and loaded into a small cup, and the spectra were observed at wavelengths ranging from 1800 to 2400 nm. Each sample was scanned five times, and the average value was recorded. The quality traits such as oleic acid, linoleic acid and oil content were analysed using NIR spectrophotometer by taking three biological replicates. Statistical significance for the difference of above biochemical parameters were calculated through student's t test at 5% level of significance.

Biometrical traits

Observations were recorded for plant height, number of primary and secondary branches, pod length, pod width, hundred pod weight, hundred seed weight and pod yield per plant on individual plants of the BC_2F_2 population. Three random sets of 10 pods / seeds were taken and multiplied by 10 and their mean was considered as hundred pod weight and seed weight, respectively. Pod yield per plant was recorded by weighing the total number of pods of each plant and expressed it in gram/plants.

Results

Development and confirmation of the F_1, BC_1F_1 and BC_2F_1 generations

The parents were genotyped with the allele-specific primers, F435SUB-R and F435-F. The primer pair amplified the mutant allele in the donor parent at 203 bp indicating the presence of *ahFAD2A* allele, whereas in the recurrent parent it was not amplified (Fig. 2). Hybridization was performed, and a total of 49 pods were obtained in *Rabi* 2021. Due to several external factors, only sixteen plants germinated in the next season (*Summer*, 2022). These sixteen plants were subjected to foreground selection, and a total of five plants were found to be heterozygous for the target *ahFAD2A* gene (Fig. 2). The details of the number of plants chosen in each generation are given in Table 1.

The confirmed F_1 plants were tagged and used as the pollen source to generate the BC_1F_1 generation by crossing with the recurrent parent TMV7. A total of 38 pods were obtained, of which only 15 plants were established in the next season (*Kharif*, 2022). Foreground selection was carried out using the same allele-specific primers, and the plants were confirmed for the presence of the *ahFAD2A* gene. Of the 15 plants, three were found to be heterozygous (Fig. 3) and were tagged for the next round of crossing to generate BC_2F_1. The confirmed plants were backcrossed with the recurrent parent, a total of 52 pods were obtained, and approximately 31 plants germinated in the next season (*Rabi*, 2023). These plants were subjected to foreground conditions, and a total of eight plants were confirmed. These plants were tagged and selfed to develop the BC_2F_2 population.

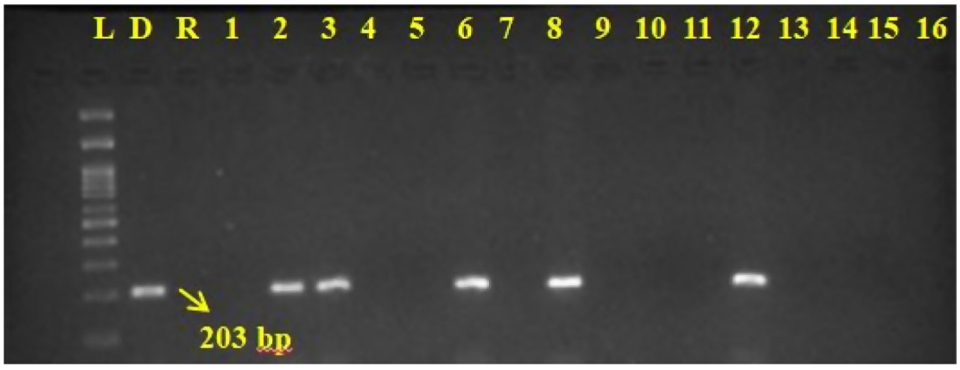

Fig. 2 Foreground selection in the F_1 population for *ahFAD2A* allele: L – 100 bp ladder, D – ICG 15419 (donor), R – TMV 7 (recurrent), 1–16 F_{1s}

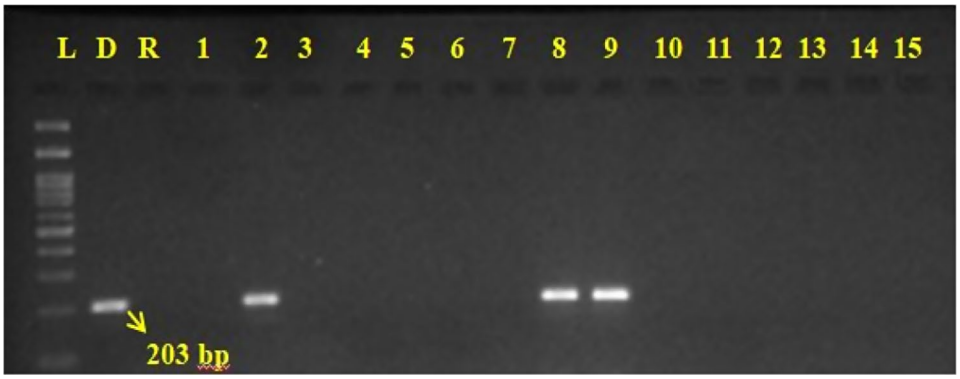

Fig. 3 Foreground selection in the BC_1F_1 population for *ahFAD2A* allele: L – 100 bp ladder, D – ICG 15419 (donor), R – TMV 7 (recurrent), 1–15 BC_1F_{1s}

Table 1 Confirmation of plants based on Foreground Selection using allele-specific primers

S.No.	Generation	Number of pods obtained	No. of plants germinated	No. of plants positive for *ahFAD2A*
1	F_1	49	16	5
2	BC_1F_1	38	15	3
3	BC_2F_1	52	31	8
4	BC_2F_2	171	123	78

Foreground selection and detection of homozygosity in the BC_2F_2 population

A total of 171 BC_2F_2 selfed seeds were obtained from the BC_2F_1 plants. Of 171 plants, only 123 germinated in the next season (*Kharif*, 2023). These 123 plants were genotyped using allele-specific primers, and a total of 45 wild-type plants and 78 plants positive for the *ahFAD2A* gene were obtained. As the allele-specific primers could detect only the presence or absence of the gene and could not differentiate between the homozygous and heterozygous conditions, a codominant marker such as CAPS marker was used to confirm the homozygosity of the *ahFAD2A* gene. The whole population was subjected to CAPS marker analysis, which included polymerase chain reaction with the primers a19F and 1056R with an expected band size of 826 bp (Fig. 4), followed by restriction digestion (Fig. 5; Additional File 1). Among the 78 positive plants, ten plants harbored the *ahFAD2A* gene under homozygous conditions. These 10 confirmed homozygous (for *ahFAD2A* mutation) plants were subjected to background selection to estimate the recovery percentage of the recurrent parent.

Recovery of the recurrent parent genome

Identification of polymorphic markers between the recurrent and donor parents

A total of 126 SSR markers spanning the complete genome were employed in this study to estimate polymorphisms between the recurrent and donor parents. Among the 126 markers, 50 were found to be polymorphic, ranging between 2 and 5 per linkage group, and the details of the polymorphic markers in the ten linkage groups of the A genome and the B genome are listed in Table 2. The polymorphism percentage between the parents was 39.7%.

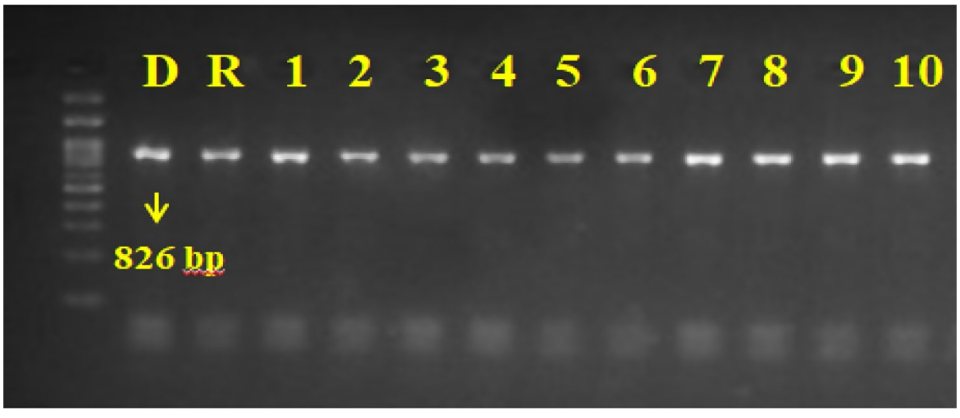

Fig. 4 Genotyping of the BC_2F_2 population with CAPS primer before digestion

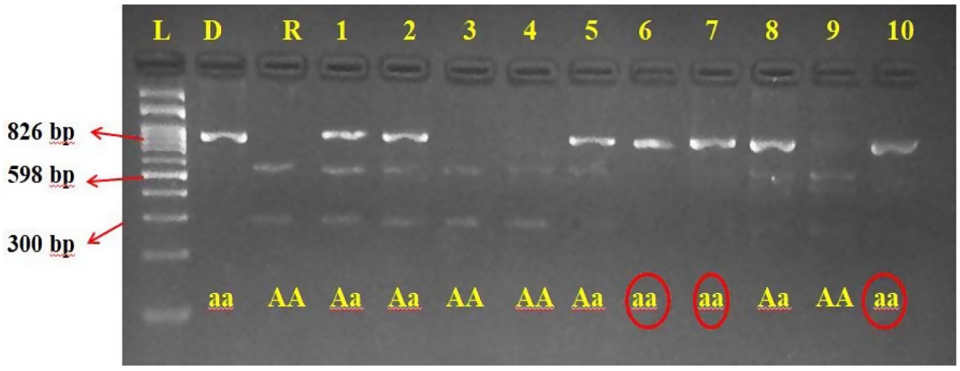

Fig. 5 Restriction digestion of *ahFAD2A* allele in the BC_2F_2 population

Screening of the polymorphic primers and estimation of the percentage of recurrent parent genome recovery in the selected lines of BC_2F_2

The recovery percentage was calculated by genotyping the ten homozygous (for *ahFAD2A* mutation) selected lines of BC_2F_2 with polymorphic primers, and the score was given as 'AA' for the presence of a recurrent parent and 'BB' for the presence of a donor parent. The genome recovery was computed using GGT, and the GGT image of chromosome number 9 of the A genome with the introgressed donor segments is given in Fig. 6. The progenies showed an average of 88.14% recovery of the recurrent parent genome, and the recurrent parent genome recovery percentage ranged from 86.22% in line no. 77 to 91.12% in line no. 23 (Table 3) representing the highest recovery of the recurrent parent genome (Fig. 7; Additional file 2).

Performance of the selected lines

The traits, plant height, number of primary branches, number of secondary branches, pod length, pod width, hundred pod weight, hundred seed weight, oil content, oleic acid content, linoleic acid content and pod yield per plant of the homozygous lines of the cross between TMV 7 and ICG 15419 were evaluated along with those of their parents (Table 4). The oleic acid content exhibited significant differences, ranging from 54.23 to 57.72%, whereas it was only 42.31% in the recurrent parent, TMV 7. Additionally, the linoleic acid content was found to be lower, with a range of 23.12–25.28%, which was less than the linoleic acid content of the parent TMV 7, which was 38.19%, thereby improving the O/L ratio to a value greater than 2, which was higher than that of the recurrent parent, which possessed an O/L ratio of just 1. Phenotypic evaluation of the selected lines in the BC_2F_2 population revealed that the line IL-68 had increased oleic acid content, decreased linoleic acid content and O/L ratios of 57.72%, 23.12% and 2.5, respectively, whereas the line IL-23 had the highest recurrent parent genome recovery of 91.12% with an oleic acid content of 55.66%.

Discussion

Groundnuts are an important source of plant-based protein, making them valuable for vegetarians and those following vegan diets. Additionally, groundnut oil is popular because of its mild flavor, high smoke availability, and versatility in various cooking methods. In India, which is a significant producer and consumer of groundnut oil, it is essential to periodically improve the quality of groundnut oil according to consumer preferences, growing health awareness and export-import trends.

The oilseed crop groundnut is rich in both monounsaturated fatty acids and polyunsaturated fatty acids, mainly oleic acid and linoleic acid, respectively. Compared with saturated fatty acids, unsaturated fatty acids are much better, but excessive and regular consumption of cooking oils rich in linoleic acid has a negative impact. The greater the degree of unsaturation is, the greater the susceptibility to oxidation, which eventually leads to the production of oxidized metabolites and contributes to a variety of chronic illnesses, such as cardiovascular diseases and Alzheimer's disease, and excessive deposition of oxidized compounds by low-density lipoprotein (LDL) can cause plaque formation in arterial walls [20]. Additionally, the half-life of this fatty acid is more than two years, so its effects can persist in the human system for a longer time [21].

Therefore, an attempt was made to reduce the proportion of polyunsaturated fatty acids, particularly linoleic acid in an agronomically superior variety TMV 7 by introgressing a gene from ICG 15419, a medium oleic donor by marker assisted backcross breeding. Despite the availability of several alternatives for oils with high oleic acid, the demand for groundnut oil remains high due to its affordability, making it accessible to a large group of people. A study was reported which attempted to introgress disease resistance in a rice genotype by both marker-assisted backcrossing and transgenics resulting

Table 2 List of polymorphic primers for the 20 linkage groups of groundnut

S.No.	Chromosome	Primer	Forward primer	Reverse primer
1	A01	Ah3TC20E08	AGGCGGGACAAAGATTACATTA	AAACTGGTGGCCAAAGCTATAA
2	A01	TC2D06	AGGGGGAGTCAAAGGAAAGA	TCACGATCCCTTCTCCTTCA
3	B01	GM1971	TTTTCTCCGAACCTTCCTTTC	AAGAAAAGAAGAGCAGCCACA
4	B01	Ah3TC20D05	CAGCACCACATGATTGTCTTTA	GATCAAACCCTCCATAATCGTA
5	A02	Ah3TC28B01	ATTTATTGCCAAATCTGTCGCT	CATTGCCAACTGTTACTACCCA
6	A02	PM32	AGTGTTGGGTGTGAAAGTGG	GGGACTCGAAACAGTGTTATC
7	A02	TC4F12	GATCTTTCCGCCATTTTCTC	GGTGAATGACAGATGCTCCA
8	B02	seq18E07	AACGTGCGTGGAAAGAGTTC	TGAGAGTGGTTTTTGTTGGTG
9	B02	seq2F05	TGACCAAAGTGATGAAGGGA	AAGTTGTTTGTACATCTGTCATCG
10	A03	Ah1TC4G02	GATCCAACTGTGAATTGGGC	CACACCAGCAACAAGGAATC
11	A03	GM1954	GAGGAGTGTGAGGTTCTGACG	TGGTTCATTGCATTTGCATAC
12	A03	IPAHM103	GCATTCACCACCATAGTCCA	TCCTCTGACTTTCCTCCATCA
13	A03	PM434	TTCGGCTGACAGCTCTAAG	GAAAGAAATTATACACTCCAATTATGC
14	B03	GM2079	GGCCAAGGAGAAGAAGAAAGA	GAAGGAGTAGTGGTGCTGCTG
15	B03	GM1536	AAAGCCCTGAAAAGAAAGCAG	CAACCAGCTCCTTCTCTACCC
16	B03	GM2301	GTAACCACAGCTGGCATGAAC	TCTTCAAGAACCCACCAACAC
17	B03	GM2009	CAAACGCATACACCCCATAAC	TTTGGTTCTCGTTTGTGTTTT
18	A04	PM375	CGGCAACAGTTTTGATGGTT	GAAAAATATGCCGCCGTTG
19	A04	GM2638	ATGCTCTCAGTTCTTGCCTGA	CAGACATAACAGTCAGTTTCACC
20	B04	seq17F06	CGTCGGATTTATCTGCCAGT	AGTAGGGGCAAGGGTTGATG
21	B04	TC11H06	CCATGTGAGGTATCAGTAAAGAAAGG	CCACCAACAACATTGGATGAAT
22	B04	GM1089	TTGGAACAAGGATGGAAAGAA	GTTTACGGTTGGCTTGTCAAA
23	A05	Ah3TC28A12	TTGAAAGCGAGAGTTTTGAGAA	TCTCAGTTTCTTTGTCGCTCAT
24	A05	GM2078	TCATGATGCAATGATAATAGGC	CTGGTCCATTGGGGACTCT
25	B05	GM1641	ACACGTGTCCCTCAAACACA	GTTGCAGAGCTCATCAAGCA
26	B05	PM50	CAATTCATGATAGTATTTTATTGGACA	CTTTCTCCTCCCCAATTTGA
27	A06	TC1A01	TCAACGCGACACAAGAAGTC	GTCGGTAAATCCGACGAAAA
28	A06	TC7C06	GGCAGGGGAATAAAACTACTAACT	TTTTCCTTCCTTCTCCTTTGTC
29	B06	Ah3TC24B05	ATTGATACCTCTTTGCTCTCGC	TGAAACCCTAACTAGCTCGGAA
30	B06	GM1991	GAAAATGATGCCGAGAAATGT	GGGGAGAGATGCAGAAAGAGA
31	B06	PM137	AACCAATTCAACAAACCCAGT	GAAGATGGATGAAAACGGATG
32	A07	Seq5D5	AAAAGAAAGACCTTCCCCGA	GCAGGTAATCTGCCGTGATT
33	A07	GM1937	TTCATCCTCTGCTTCCTTTGA	TGACCAAACCCATCATCATCT
34	B07	GM2605	ACTGCTGCCATGGTTGAGTTA	TTTCGCACTTTCTCAGTTTCC
35	B07	TC3B05	GGAGAAAACGCATTGGAACT	TTTGTCCCGTTGGGAATAGT
36	A08	GM1760	TGAAGAGCCATGTCAGATCG	AGGGCCCCAACAAGATAAGT
37	A08	GM1863	CACACCCAGTCACTCTCTCTG	TCTGATGTTCTGTGTGTGGAGA
38	B08	GM2504	ACATCAATCCCTGCCTACCTC	TCGGATTCTGTTACCACCTCA
39	B08	Ah3TC20B05	GCATGTAAACTATGCAATCGCT	CAACAACTTATTCCACCAAATATCA
40	B08	IPAHM406	TGAAAGGGATTGGACCAAAA	TGTTGGACAGGATTTCACACA
41	A09	Ah3TC25B04	TGCTTGTGTATTGAGCTGTCCT	CATCTGCCAAGGTCCTAAAATC
42	A09	GM1911	CAGCTTTCTTTCAATTCATCCA	CACTTCGTGTTCTTCCTGCTC
43	A09	F435-SUB	ATCCAAGGCTGCATTCTCAC	TGGGACAAACACTTCGTT
44	A09	seq8D9	TGAGTTTCCCCAAAAGGAGA	CAACAACAATACGGCCAACA
45	B09	Lec1	CAAGCATCAACAACAACGA	GTCCGACCACATACAAGAGTT
46	B09	seq14H6	GCAACTAGGGTGTATGCCGT	CAACCCTATACACCGAGGGA
47	A10	GM2605	ACTGCTGCCATGGTTGAGTTA	TTTCGCACTTTCTCAGTTTCC
48	A10	ARS773	GGGAACGAATGAAGTAGGCA	GCATGGGTTTCAAGGTCTGT
49	B10	TC7H11	AGGTTGGAACTATGGCTGATTG	CCAGTTTAGCATGTGTGGTTCA
50	B10	Ah3T23H10	TCCCTTTGAGTCATTCATTGTG	CATCAGAGCTCCTTTTCCCTAA

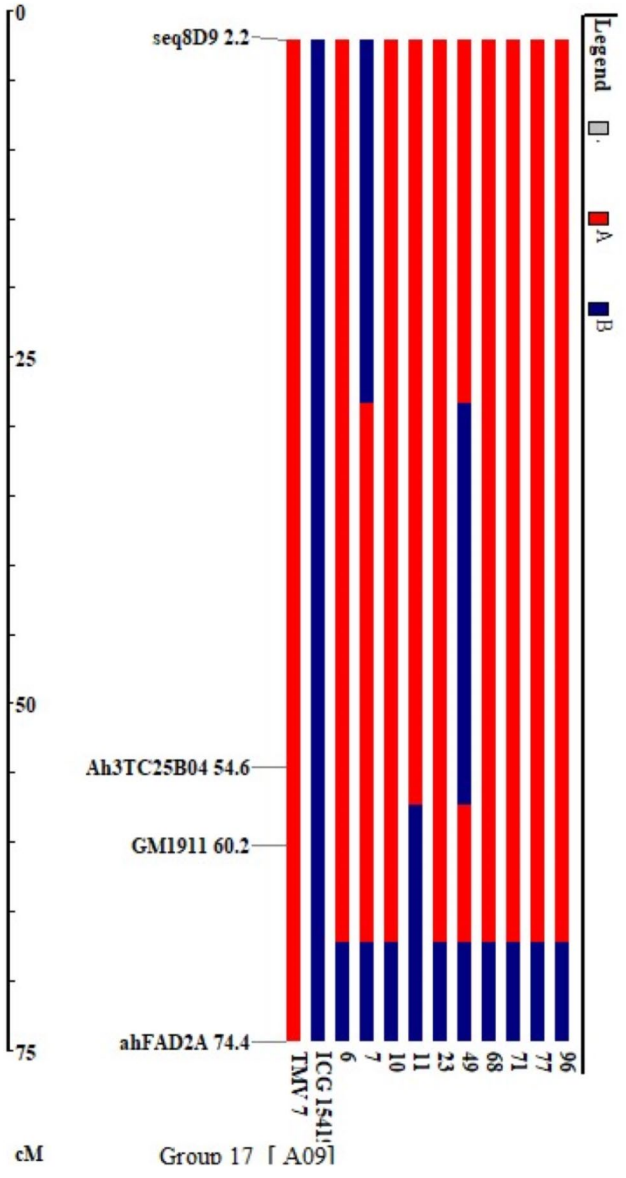

Fig. 6 GGT image of the chromosome number 9 of the A genome with the introgressed gene

Table 3 Recovery percentage of the recurrent parent genome in the elite BC_2F_2 lines of the cross between TMV 7 and ICG 15419

S.No.	Line No.	Recovery %
1	IL – 6	90.14
2	II – 7	88.18
3	IL – 10	86.72
4	IL – 11	85.24
5	IL – 23	91.12
6	IL – 49	89.16
7	IL – 68	87.20
8	IL – 71	90.14
9	IL – 77	86.22
10	IL – 96	87.31
Mean		88.14

Table 4 Phenotypic evaluation of the pod-related traits in the selected lines of BC_2F_2

Introgressed Lines	PH	PB	SB	PL	PW	HPW	HSW	OIL	OA	LA	O/L	PYP
IL – 6	34.7	7	8	2.43	1.33	74.26	26.97	52.36	55.57	24.71	2.25	16.87
II – 7	34.1	8	14	2.23	1.10	72.84	27.63	51.60	54.34	25.28	2.15	12.31
IL – 10	35.8	5	11	2.67	1.27	68.84	23.08	49.00	55.06	24.21	2.27	27.26
IL – 11	36.7	9	14	2.63	1.10	76.92	28.57	52.61	54.23	25.34	2.14	29.41
IL – 23	33.5	7	13	2.23	1.00	76.19	34.38	52.36	55.66	24.22	2.31	16.81
IL – 49	36.6	8	15	2.43	1.37	72.67	25.45	50.78	56.02	23.24	2.41	24.73
IL – 68	35.4	8	13	2.47	1.23	76.92	26.79	50.19	57.72	23.12	2.50	27.34
IL – 71	34.3	10	12	2.47	1.33	64.52	26.42	53.30	54.36	24.38	2.23	26.29
IL – 77	35.2	9	15	2.50	1.30	68.97	25.93	52.81	55.43	24.76	2.24	22.67
IL – 96	32.7	9	4	2.73	1.07	75.71	29.93	46.85	54.87	24.36	2.25	19.41
TMV 7	32.43	7	12	2.33	1.37	80.37	32.94	48.03	42.31	38.19	1.11	23.12
ICG 15419	53.02	5.89	11	4.72	1.52	91.25	62.56	47.51	60.72	21.57	2.82	22.92

(PH - Plant height, PB - Number of primary branches, SB - Number of secondary branches, PL - Pod length, PW - Pod width, HPW - Hundred pod weight, HSW - Hundred seed weight, OIL - Oil content, OA - Oleic acid content, LA - Linoleic acid content, O/L - oleic acid/linoleic acid ratio and PYP - Pod yield per plant)

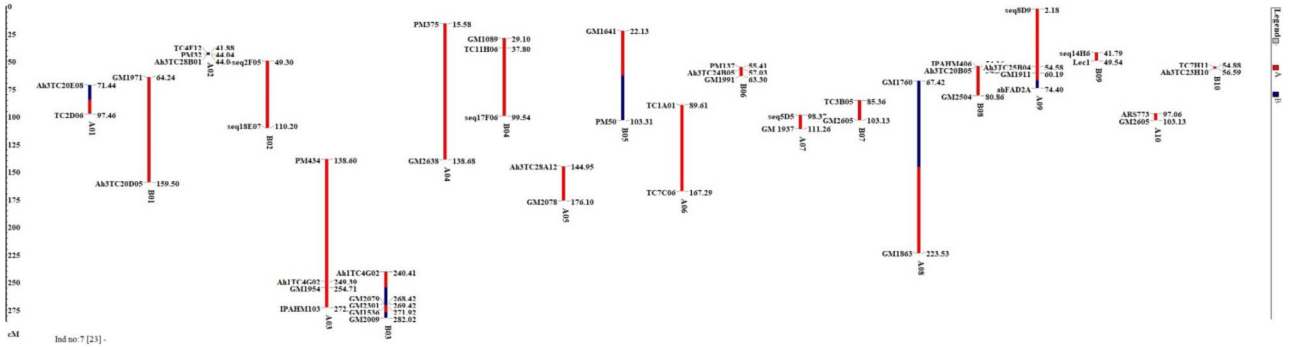

Fig. 7 GGT image of the line with highest recurrent parent genome recovery

in similar outcomes [22]. Hence, by considering the efficiency and precision of marker-assisted backcross breeding, present investigation was carried out to introgress high oleic content in elite variety through MABC.

In this study, allele specific markers were employed in screening progenies for the presence of substitution mutations in the A genome and the identified five positive plants in the F_1 generation were backcrossed with the recurrent parent to give rise to BC_1F_1. Similar studies to confirm the presence of mutation in the crosses GPBD 4 and G 2–52 with SunOleic 95R employed the same foreground marker [23]. Another report by [13] performed the transfer of *ahFAD2A* allele in to the background of GJG 9, GG 20 and GJGHPS 1 to improvise the oleic content of the widely accepted cultivars. Further experiments by [11, 24, 25] led to the development of high oleic lines in the genetic background of different popular cultivars in India.

The three positive plants in the BC_1F_1 generation were backcrossed with the recurrent parent to develop BC_2F_1. The recurrent parent genotype can be easily recovered with 2 to 3 backcrosses by marker assisted backcross breeding than the traditional method [26]. In BC_2F_2, the utilization of CAPS markers differentiated the homozygous and heterozygous lines based on the banding pattern obtained after digesting the PCR products with the restriction enzyme *Hpy99I.* The introgressed lines showed that the mutant allele of *ahFAD2A* was intact, while the wild type had a recognition site for digestion by the enzyme, resulting in two fragments of 598 bp and 228 bp [17]. A similar study was reported incorporating CAPS marker to identify homozygous lines of the cross ICGV 05141 and Sun Oleic 95R [8]. CAPS marker were also employed in the marker assisted introgression of two popular groundnut varieties, GG-7 and TKG-19 A by crossing it with a high oleic donor [27].

Background selection is typically carried out with one to three markers in each chromosome to estimate the recovery of the recurrent parent genome [28]. In this study, despite the use of numerous prescreened markers to identify polymorphisms between the two parents, the parental polymorphism was just 39.7% which was very low due to polyploidization, as reported by [29] and [30]. A maximum of 91.12% of recurrent parent genome recovery in the BC_2F_2 generation explains that the recurrent parent contributes more to the genetic background of the homozygous lines except for the desired segment acquired from the donor. Though additional introgression of donor segments was observed in A01, A02, B03, B05 and A08 in IL-23 but fortunately the biometrical evaluation of the introgressed lines displayed an agronomic performance similar to the recurrent parent assuming that the linkage drag has not disturbed the performance of these introgressed lines. It was also inferred that no significant differences were observed in traits other than oleic acid content, linoleic acid content or the O/L ratio between the introgressed homozygous lines and the recurrent parent. Furthermore, the presence of the *ahFAD2A* gene from the donor on the ninth chromosome of all ten homozygous lines coupled with an increase in the oleic acid content confirmed the successful marker assisted introgression.

Consistent seed size and oil content are the distinct characteristics of the variety, TMV 7 which is specific to Tamil Nadu, India. The improved version of the variety posessing an increased storage life would be an added advantage amidst other commercial varieties under cultivation. Additionally, improving vegetable oils with increased oleic acid can also be used to produce biodiesel due to its unique chemical properties and composition [31]. Such oils tend to have more polarity than mineral oil, thereby forming an even film on the surface of the metal, causing good lubrication and less friction [32]. On the other hand, it is a renewable alternative and reduces the dependency on natural resources, which are on the verge of depletion and minimize the impact on the environment.

Conclusion

The BC_2F_2 population developed from the cross between TMV 7 and ICG 15419 was subjected to foreground selection, CAPS marker analysis and background

selection. A total of ten homozygous individuals confirmed by molecular genotyping were evaluated for biometric traits, especially oleic acid and linoleic acid content. The line IL-68 was found to contain 57.72% oleic acid, with a recurrent parent genome recovery of 87.20%. The highest recurrent parent genome recovery was observed for IL-23 (91.12%), which contained 55.66% oleic acid. As the goal of this investigation was to improve the oleic acid content of the variety TMV 7, the line IL-23 was developed as the improved version of TMV 7.

Abbreviations

A	Adenine
BC_1F_1	Back Cross 1 Filial 1
BC_2F_1	Back Cross 2 Filial 1
BC_2F_2	Back Cross 2 Filial 2
Bp	Base Pair
cM	Centi Morgan
CAPS	Cleaved Amplified Polymorphic Sequences
C	Cytosine
°C	Degree Celsius
DNA	Deoxyribo Nucleic Acid
DP	Donor Parent
EDTA	Ethylenediamine Tetraacetic Acid
FAD	Fatty Acid Desaturase
F_1	Filial 1
F	Forward
GGT	Graphical Genotypes
G	Guanine
IC	Internal Control
MABC	Marker Assisted Back Cross Breeding
MAB	Marker Assisted Breeding
MAS	Marker Assisted Selection
MUFA	Mono unsaturated fatty acids
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
RP	Recurrent Parent
RPGR	Recurrent Parent Genome
R	Reverse
SSR	Simple Sequence Repeats
SUB	Substitution
TNAU	Tamil Nadu Agricultural University
T	Thymine
TE	Tris EDTA Buffer
UV	Ultra Violet
UFA	Unsaturated Fatty Acid

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05387-9.

Supplementary Material 1

Supplementary Material 2

Author contributions

RLV conducted the research experiments and wrote the manuscript. SS, JH and MPK helped in conducting the experiments. AP and SS designed the study and supervised it. KP and SK helped with the statistical analysis and interpretation. AP and SS helped in genotype collection and corrected and revised the manuscript.

Funding

We express our gratitude to the Science and Engineering Research Board, Department of Science and Technology, India, for their financial support.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 13 April 2024 / Accepted: 5 July 2024
Published online: 31 July 2024

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