QTL mapping of soybean oil content for marker-assisted selection in plant breeding program

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ABSTRACT. The present study was undertaken to detect and map the quantitative trait loci (QTL) related to soybean oil content. We used 244 progenies derived from a bi-parental cross of the Lineage 69 (from Universidade Estadual Paulista "Júlio de Mesquita Filho"/Faculdade de Ciências Agrárias e Veterinárias - Breeding Program) and Tucunaré cultivar. A total of 358 simple sequence repeat (SSR; microsatellite) markers were used to investigate the polymorphism between the parental lines, and for the polymorphic lines all the F_2 individuals were tested. Evaluation of the oil content and phenotype was performed with the aid of a Tango equipment by near infra-red reflectance spectroscopy, using single F_2 seeds and $F_{2:3}$ progenies, in triplicate. The data were analyzed by QTL Cartographer program for 56 SSR polymorphic markers. Two oil-content related QTLs were detected on K and H linkage groups. The total phenotypic variation

explained by QTLs ranged from 7.8 to 46.75% for oil content. New QTLs were identified for the oil content in addition to those previously identified in other studies. The results reported in this study show that regions different from those already known could be involved in the genetic control of soybean oil content.

Key words: *Glycine max*; SSR; NIR; Molecular markers

INTRODUCTION

Soybean has a well-established supply chain and wide application throughout Brazil, mainly due to the rapid and continuous progress in breeding programs that are underway across the country, which enabled the effective production of the crop all over the country, contributing to a breakdown of agricultural boundaries.

Through the advances in breeding programs in recent decades, several technologies have been incorporated to affect the delivery of new cultivars. Thus, use of molecular marker has the potential for identifying genes of agronomic interest in the breeding programs.

The vast majority of the economically important traits are of quantitative nature. Those traits of which the phenotypic expression displays continuous variation are attributed to simultaneous segregation of many genes within the genome. Chromosomal regions defining the location of such genes are defined as quantitative trait loci (QTLs).

Microsatellite or simple sequence repeat (SSR) marker is a sequence with one to six base pairs repeated in tandem. SSRs are multiallelic, co-dominant, and are easily detected by polymerase chain reaction (PCR); they are relatively abundant, have extensive genome coverage, and require a low amount of DNA for amplification (Powell et al., 1996).

Linkage genetic map construction through molecular markers can support strategies to be incorporated into the breeding programs to estimate which (and how many) QTLs are responsible for trait variation and to locate their position within the genome, as well as to evaluate their effects and inter-relationships. Development of microsatellite markers in soybean has been carried out for use in genetic mapping. These markers enable candidate genes to be linked to agronomic traits of interest. These markers are considered ideal for assisted selection process because of their benefits (Cato et al., 2001).

Thus, a direct application of genetic map construction is to locate economically important genes or QTLs. Furthermore, genetic mapping allows marker-assisted selection (MAS) in plant breeding programs involving selection, thereby, reducing time and resources spent in developing new cultivars.

Few reports have already described QTLs for soybean oil content using different markers, plant population types and sizes, as well as exotic and adapted genotypes (Panthee et al., 2005; Nichols et al., 2006; Shibata et al., 2008).

With the advent of molecular markers, it became possible to identify such QTL regions that affect quantitative traits. Therefore, mapping a QTL means to detect its position within the genome and to estimate its genetic effects in the adopted model, such as being additive and dominant, among others (Toledo et al., 2008).

Responding to the market demand for soybean cultivars with high oil content, breeding programs focus on the development of varieties with good agronomic traits, early cycle, and high

oil content to replace the obsolete existing ones. This fact is of great importance, especially in areas with sugarcane cultivation, where soybean could be an option for growing in reform areas as well as for biodiesel production or industrial use.

The present research was, therefore, undertaken to detect the quantitative trait loci associated with soybean oil content in $F_{2:3}$ populations derived from the cross between early lineages and those adapted for sugarcane-soybean rotation (having low oil content) as well as genotypes not adapted to this system (having high oil content); the aim of the study was to use them in the selection of the segregating populations for soybean breeding programs.

MATERIAL AND METHODS

Growth conditions and generation of F_2 segregating populations

Artificial breeding (hybridization) was carried out under greenhouse conditions to generate new soybean populations for use in genetic mapping and for selection of new genotypes.

Parents selected for crossings showed earliness, superior agronomic traits, resistance to main soybean diseases, and good seed oil content. The genotypes (L69, L23, L54, L62, L01, L02, L52, L53, and L70) that were used as sources for precocity and agronomic superiority belonged to the breeding program from Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Jaboticabal. Two commercial cultivars with high oil content (FMT Tucunaré and UFUs Carajás) were also included in the study. During the crossings, trait contrasts were observed for greater variability exploitation (Table 1).

Table 1. List of parents used in the crossings along with a description of important traits of each genotype.

Parents	Cycle	Trait
Lineage 69	Up to 120 days (early)	Full adaptation; yield
Lineage 23	Up to 120 days (early)	Full adaptation; yield
Lineage 54	Up to 120 days (early)	Full adaptation; yield
Lineage 62	Up to 120 days (early)	Full adaptation; yield
Lineage 01	Up to 120 days (early)	Full adaptation; yield
Lineage 02	Up to 120 days (early)	Full adaptation; yield
Lineage 52	Up to 120 days (early)	Full adaptation; yield
Lineage 53	Up to 120 days (early)	Full adaptation; yield
Lineage 70	Up to 120 days (early)	Full adaptation; yield
FMT Tucunaré	125-130 days (medium)	High oil content
PI 200487 (Kinoshita)	131-140 days (medium)	SR (R)
PI 471096 (Orba)	131-140 days (medium)	SR (R)
Bragg	131-140 days (medium)	MI (R), MJ (R)
BRS Sambaíba	131-140 days (medium)	SR (MR), SB (R)
MG/BR 46 (Conquista)	131-140 days (medium)	SB (R), O (R)
IAC 17	Up to 120 days (early)	SB (R), O (R)
IAC Foscarin 31	Up to 120 days (early)	SB (MR)
BRSMG Confiança	Up to 120 days (early)	SB (R), O (R)
BRSMG Renascença	121-130 days (semi-early)	SCN (R 3), SB (R), O (R), MI (R)
BRS 231	121-130 days (semi-early)	SCN (R 3), SB (R)
FMT Matrinxã	121-130 days (semi-early)	SCN (R 1, 3), SB (R), MI(R)
FT-Cometa	Up to 120 days (early)	MI (MR), MJ(R)
IAC 23	Up to 120 days (early)	SB (R), O (R)
UFUs Carajas	115 days (early)	High oil content

R = resistance to diseases, 1 and 3 = nematode species, SR = soybean rust, MI = Mildew, O = Oidium, MJ = *Meloidogyne javanica*, SB = stem blight, SCN = soybean cyst nematode.

Artificial hybridizations were performed in an air-conditioned greenhouse in the Department of Crop Production at UNESP/FCAV, Jaboticabal, SP, Brazil. The temperature and relative humidity in the greenhouse was controlled to nearly simulate the environmental conditions of the field. Sowing was done every 7 to 10 days to synchronize the blooming among the parents; viable flower buds of different genotypes were produced to act as male and/or female parents. Five-liter pots filled with a mixture of soil, sand, and vermiculite provided the substrate. Five seeds of each genotype were sown per pot in triplicate; after thinning three plants remained in each pot. These plants were grown vertically and used for hybridization. Seeds obtained from the artificial hybridizations were selected and sown to obtain segregating populations (F_2).

Genomic DNA extraction and quantification

Total genomic DNA was extracted from the youngest trifoliate leaf tissue by the CTAB method (cetyltrimethylammonium bromide) adapted for soybean as described by Ferreira and Grattapaglia (1998). The DNA samples were spectrophotometrically quantified using a Nanodrop® (NanoDrop Technologies LLC, Wilmington, DE, USA). The DNA quality was assessed by measuring its absorbance at 260 and 280 nm.

DNA extracted from F_1 and F_2 populations was used to identify and confirm the hybridized plants for further use in the genetic mapping of QTLs for seed oil content.

Molecular analyses of F_1 population

F_1 hybrid plants were identified using SSR microsatellite markers. Amplification of microsatellite markers was performed by PCR. The PCR mixture consisted of 1X buffer (10 mM Tris-HCl, pH 8.0, 50 mM KCl), 1.5 mM $MgCl_2$, 0.15 µM each dNTP, 0.15 µM forward and reverse primers, 1 U Taq polymerase, 300 ng genomic DNA, and 25 µL ultrapure water. The amplification program consisted of an initial denaturation at 94°C for 7 min, followed by 32 cycles of denaturation at 94°C for 1 min, primer specific annealing temperature for 1 min, extension at 72°C for 2 min, and a final extension at 72°C for 7 min. The PCR product was electrophoresed at 80 V for about three hours in 1X TBE buffer on a high-resolution 3% agarose gel (UltraPure Agarose 1000). The gel was subsequently stained with 10 mg/mL ethidium bromide, visualized under UV light, and photographed using a gel-documentation system (QUANTUM-ST4).

Twenty-seven markers of different linkage groups belonging to the soybean genetic map were used in the PCRs to check the heterozygosity of individuals obtained after intercrossing. Individuals that showed a band profile of both the locus alleles indicated the effectiveness of crossing and were identified to have originated from hybridizations; these were selected for obtaining the next generation (F_2).

Molecular analyses of F_2 population

Molecular analyses of the F_2 population were performed in the Laboratory of Genetic Diversity and Plant Breeding located in the Genetics Department of Escola Superior de Agricultura “Luiz de Queiroz”, in Piracicaba, SP, Brazil.

Bulked segregant analysis (BSA) is a quick and efficient process to identify specific genomic

regions, which can be associated with particular phenotypic characteristics (Michelmore et al., 1991). For this analysis, the microsatellite loci (SSRs) were evaluated for the degree of polymorphism by comparing the genitors Tucunaré and Lineage 69. The loci with the greatest potential to generate polymorphic markers and produce new high-quality bands were selected. A 20-progeny bulk, with 10 progenies each containing high and low oil content, from the mapped population generated by crossing the Tucunaré cultivar (high oil content) and Lineage 69 (low oil content), was evaluated to assess the possible association of microsatellite markers with the oil content. After identification of the polymorphic markers, they were evaluated in the mapped population.

For amplification, the reaction mixture contained 20 ng DNA, 10 mM forward primer, 10 mM reverse primer, 10 mM fluorescent M13 primer, 25 mM each dNTP, 10X PCR buffer, 2.5 mg/mL bovine serum albumin (BSA), 25 mM $MgCl_2$, and 1 U Taq DNA polymerase enzyme in a total volume of 10 μL. Microsatellite region amplification was performed according to the following protocol: 5 min at 94°C, followed by 10 cycles of 1-min denaturation at 94°C, 1-min annealing at 50°C, and 1 min extension at 72°C; after each cycle the annealing temperature was decreased by 1°C. Thereafter, 30 cycles were performed as follows: 94°C for 40 s, 40°C for 40 s, and 72°C for 1 min; this was followed by a final extension at 72°C for 10 min.

The PCR products were purified by electrophoresis on a polyacrylamide gel under denaturing conditions and sequenced using an automated sequencer (LI-COR 4300S DNA Analysis System; LI-COR Biosciences, Lincoln, NE, USA). The number of base pairs in the alleles was inferred based on standard commercial markers (ladder) and the images were filed for subsequent allele labeling and genotyping using the SAGA Lite and the SAGA MX Generation software (LI-COR Corporate). The results were checked and, if necessary, allele identifications were manually corrected.

Polymorphism information content (PIC)

PIC was calculated using the formula proposed by Anderson et al. (1993), which takes into account the detected alleles, their frequency, and distribution over the studied population. PIC was estimated to determine the value of each marker in polymorphism detection among the soybean progenies. The formula is presented below:

$$PIC_i = 1 - \sum_{j=1}^{n} p_{ij}$$

where, p_{ij} stands for the frequency of *j* allele within the *i* marker (summation extends over all the alleles). Calculation was based on the number of detected alleles per marker for a certain locus and relative frequency of each allele in the evaluated progeny set.

Construction of the linkage map

Linkage map was constructed using the R statistical software package, OneMap (Margarido et al., 2007). The linkage groups (LG) were established by adopting a minimum logarithm of odds (LOD) of 3.84 for connection among the markers, for ordering of marker strains, and maximum recombination

fraction (r) of 0.30. The 'order.seq' function was used to determine the best order of markers within each linkage group, once the software automatically used the 'compare' and 'try.seq' functions. Distances between the markers, in centimorgans (cM), were estimated by Kosambi function.

Mapping of QTL for oil content

QTL detection and mapping analyses were carried through composite interval method (CIM; Zeng, 1994). CIM was run on model 6 of *Zmapqtl*, which was used as a variable selection model for the forward and backward regression. The presence of QTL was declared every 1 cM (centimorgan) with a 10-cM window. Minimum LOD score was established using a permutation test (1000 runs) with an overall P = 0.05. All the analyses were performed by means of a QTL cartographer, version 2.5 (Wang et al., 2012).

Oil content of the progenies

The seed oil content was obtained using a near infrared spectrometer Tango model (Bruker Co., Ltd. Germany). Spectrum readings were taken in the range between 11,536 to 3952 cm^{-1} with 64 scans, at 16 cm^{-1} resolution and 8 cm^{-1} interval. Percentage of oil content was obtained for all the F_2 seeds of the crosses. Because of F_2 being segregating populations, analyses were made in triplicate per seed unit. In the subsequent generation, the analyses were confirmed in the progenies derived from each individual plant ($F_{2:3}$ seeds); the experiment was performed in triplicate.

RESULTS

The average percentage of seed oil content estimated using triplicate NIR readings is shown in Table 2. The average seed oil content in the segregating populations of 244 progenies used for QTL mapping ranged from 14.35 to 23.53%.

Among the 358 SSRs evaluated by BSA analysis, 327 (91.34%) produced high quality band standards, of which 226 showed monomorphic strains, while 101 demonstrated polymorphism between the parents, and 10 loci presented possible relationships between high and low oil content assessed in bulk individuals. The mapped population from these genotypes was thus shown to be effective for construction of genetic maps related to soybean oil content.

From the 101 polymorphic SSRs evaluated by BSA analysis, 62 loci showed high quality bands suitable for genotyping. PIC ranged from 0.24 to 0.58 with an average of 0.37. In a similar result, Vieira et al. (2009), who analyzed soybean cultivars, detected an average PIC of 0.40.

A total of 56 markers from 17 LGs were used for F_2 genotyping based on the Soybean Consensus Map 4.0. Considering an LOD threshold of 3.5, two QTLs for the target trait were located on chromosome 9 (LG - K) and 12 (LG - H); this result was significant at $P < 0.05$ (Figure 1). The QTL identified on chromosome 9 (LG - K) accounted for 7.8% phenotypic variation, while that on chromosome 12 (LG - H) was responsible for 46.75% variation (Table 3).

Table 2. Seed oil content in 244 progenies obtained from the cross between the Tucunaré cultivar and Lineage 69, which were used for QTL mapping.

F_2	Average (%)	F_2	Average (%)	F_2	Average (%)	F_2	Average (%)	F_2	Average (%)
C23P1	20.057	C23P62	19.070	C23P158	17.910	C23P228	19.953	C23P301	18.846
C23P2	19.173	C23P63	17.888	C23P159	17.894	C23P229	19.753	C23P302	21.273
C23P3	20.647	C23P64	19.115	C23P160	17.543	C23P233	20.223	C23P303	19.626
C23P4	20.809	C23P65	21.939	C23P161	18.616	C23P234	19.233	C23P304	19.452
C23P5	20.574	C23P67	20.213	C23P162	16.934	C23P235	17.245	C23P305	18.744
C23P6	18.911	C23P68	14.601	C23P163	18.013	C23P238	19.632	C23P306	19.325
C23P7	20.762	C23P70	15.810	C23P164	16.496	C23P240	17.982	C23P307	19.841
C23P8	20.986	C23P71	21.582	C23P165	16.394	C23P241	18.173	C23P308	20.894
C23P9	18.920	C23P72	19.071	C23P166	19.624	C23P242	19.647	C23P309	20.637
C23P10	22.435	C23P75	19.777	C23P167	18.685	C23P243	20.456	C23P310	20.751
C23P11	19.517	C23P77	20.342	C23P168	18.290	C23P248	15.643	C23P311	19.130
C23P12	22.529	C23P79	20.531	C23P169	18.762	C23P251	21.932	C23P312	19.257
C23P13	22.275	C23P80	18.631	C23P170	17.066	C23P252	23.166	C23P313	18.316
C23P14	20.341	C23P81	20.576	C23P172	17.093	C23P254	19.960	C23P314	18.819
C23P15	17.149	C23P82	19.704	C23P173	19.523	C23P255	18.770	C23P315	19.462
C23P16	18.307	C23P83	21.238	C23P175	19.089	C23P256	19.475	C23P316	19.690
C23P20	21.120	C23P84	17.222	C23P177	17.733	C23P257	17.459	C23P318	18.703
C23P21	17.132	C23P86	16.995	C23P178	19.110	C23P261	20.343	C23P319	17.063
C23P22	19.145	C23P87	20.147	C23P181	18.646	C23P262	18.121	C23P320	17.777
C23P23	20.250	C23P88	18.848	C23P182	17.78	C23P263	20.324	C23P321	17.014
C23P24	20.090	C23P93	19.415	C23P183	18.843	C23P264	18.375	C23P322	17.549
C23P25	20.901	C23P96	18.084	C23P185	18.641	C23P265	19.872	C23P323	18.668
C23P26	21.660	C23P97	19.167	C23P186	18.299	C23P266	20.864	C23P324	19.517
C23P27	20.673	C23P99	23.344	C23P188	20.773	C23P267	19.616	C23P325	19.039
C23P28	20.977	C23P101	18.614	C23P189	17.279	C23P268	19.779	C23P326	19.846
C23P29	22.864	C23P102	21.664	C23P191	19.738	C23P271	19.566	C23P327	20.601
C23P31	20.007	C23P114	19.467	C23P192	15.782	C23P272	20.835	C23P328	19.397
C23P32	21.398	C23P116	19.017	C23P194	14.820	C23P273	19.797	C23P329	18.811
C23P33	20.039	C23P122	18.640	C23P197	19.166	C23P274	20.524	C23P330	19.691
C23P34	21.348	C23P124	16.765	C23P198	18.782	C23P275	19.557	C23P331	19.779
C23P35	19.271	C23P125	23.232	C23P199	14.549	C23P277	19.720	C23P332	14.945
C23P36	17.851	C23P127	20.99	C23P201	18.604	C23P281	20.524	C23P334	20.445
C23P37	17.991	C23P129	20.800	C23P202	19.228	C23P282	19.169	C23P336	17.286
C23P38	21.763	C23P131	19.227	C23P203	17.690	C23P284	19.240	C23P337	19.336
C23P39	18.825	C23P133	19.837	C23P206	19.630	C23P285	20.378	C23P338	17.792
C23P40	23.531	C23P134	19.493	C23P209	16.942	C23P286	19.595	C23P341	19.972
C23P41	18.048	C23P135	19.959	C23P210	19.398	C23P287	20.688	C23P344	18.559
C23P42	19.600	C23P137	22.349	C23P212	19.062	C23P288	19.884	C23P345	15.399
C23P43	20.159	C23P140	19.121	C23P215	20.735	C23P289	18.525	C23P346	14.406
C23P44	19.277	C23P144	17.988	C23P216	18.434	C23P290	20.428	C23P347	17.865
C23P45	18.832	C23P145	23.477	C23P217	19.285	C23P291	19.228	C23P348	19.097
C23P46	17.308	C23P146	21.312	C23P218	19.694	C23P292	19.679	C23P349	20.659
C23P47	18.979	C23P147	14.349	C23P219	18.229	C23P293	17.866	C23P351	21.052
C23P49	18.938	C23P148	17.705	C23P220	21.358	C23P295	19.009	C23P352	19.330
C23P50	21.221	C23P151	17.638	C23P222	17.443	C23P296	21.513	C23P355	17.320

Continued on next page

Table 2. Continued.

F_2	Average (%)	F_2	Average (%)	F_2	Average (%)	F_2	Average (%)	F_2	Average (%)
C23P55	22.447	C23P152	17.061	C23P224	18.645	C23P297	18.625	C23P357	21.305
C23P56	20.680	C23P153	17.157	C23P225	17.780	C23P298	17.927	C23P363	18,336
C23P57	17.004	C23P154	19.460	C23P226	18.982	C23P299	19.382	C23P30	19.660
C23P58	19.425	C23P157	17.057	C23P227	18.838	C23P300	19.409		

C = crossing; P = plant.

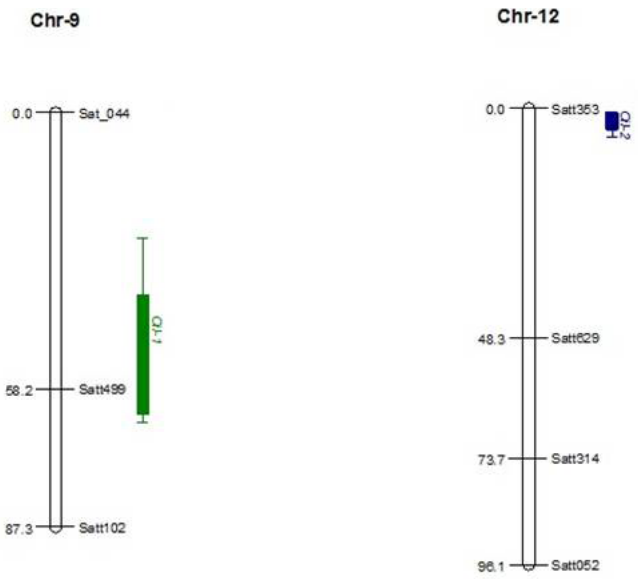

Figure 1. Linkage groups of the F_2 population consisted of 244 progenies of soybean generated by crossing Lineage 69 and the Tucunare cultivar, and LOD of 3.84 was used as a criterion. To the left are the distances between the markers, and to the right are the identifications.

Table 3. QTLs for soybean seed oil content evaluated in 244 progenies obtained from the crossing of Lineage 69 and the Tucunaré cultivar.

Chromosome	LG	Position (cM)	Markers	R^2 (%)
Chr-9	K	41-55	Sat_044 - Satt499	7.8
Chr-12	H	0-14	Satt353 - Satt629	46.75
Total				54.55

LG = linkage group; cM = centimorgans; R^2 = recombination frequency.

DISCUSSION

Previous studies have demonstrated the existence of QTLs for soybean oil content at different regions along the soybean genome. Several authors (Mansur et al., 1996; Brummer et al., 1997; Specht et al., 2001) have reported other quantitative loci with different magnitudes, locations, and genetic populations.

Others researchers (Sebolt et al., 2000; Csanádi et al., 2001; Chung et al., 2003; Reinprecht et al., 2006) have also demonstrated the genomic regions involved in the control of oil content in different soybean populations. These observations validate the association of markers with the trait assessed in the present study.

Association of LG I with this trait has been previously reported by several authors. Chung et al. (2003) mapped the QTLs that explained 27.8 and 24.2% of the variation in oil content in the years of 1996 and 1999, respectively. Tajuddin et al. (2003) used 157 RILs and mapped QTLs at the Satt239 region to explain an 8.6% variation in the oil content variation over a period of two years.

Panthee et al. (2005) detected QTLs for oil and protein contents in a population of 101 RILs derived from crosses between N98-984 and TN93-99. Among the 585 SSRs tested by these authors, 94 markers were polymorphic in the evaluated population. One QTL in LG G accounted for about 20% of the protein content and three QTLs in D1b, G, and O linkage groups explained 11.8, 9.4, and 15.0% of the oil content in this population.

Moreover, Nichols et al. (2006) studied different populations of $RC_4F_{3:4}$ and RC_5F_5 isoline for oil and protein contents using AFLP and SSR markers of LG I. In the $RC_4F_{3:4}$ populations, these authors mapped one QTL for protein and oil content within 11 cM interval between Satt614 and Satt354. When analyzing the RC_5F_5 populations, the location of this QTL was redefined to 3 cM interval between Satt239 and ACG9b; this accounted for 15.4, 11.7, 17.7, and 21.1% variation in the protein content for each RC_5F_5 population.

Oil content related loci at Satt239 LG I were reported by several authors in different genetic backgrounds and environments. This indicates conserved genes within the region, thereby, validating the presence of this locus in the genome of species; this has a great importance for the genetic mapping of oil content to accurately determine the QTL location. Furthermore, the same intervals that revealed the pleiotropic effects of one or more genes in the region also affected the protein content.

A genetic study of oil and protein contents in *Glycine max* x *Glycine soja* revealed normal distribution and high heritability in F_2 and recombinant populations; moreover, there was a negative correlation between such characters. A QTL for lipid content and concentration of linoleic acid was detected within the same position near Satt384 SSR marker in L, G, and E groups, suggesting that those QTLs responsible for linoleic acid can simultaneously control the lipid content partially (Shibata et al., 2008).

Within the LG A1, between Satt200 and Satt225 strains, the QTL for oil content was detected, which accounted for 17.3% of the trait variation. In addition, the Satt236 marker associated with oil content explained 5.2% variation (Rodrigues et al., 2010).

Rodrigues et al. (2010) studied 207 progenies obtained from the crosses between Lineage CS3032PTA276 and UFS2012 variety. These authors evaluated 357 SSR loci pairs for polymorphism of parent populations; of these about 100 loci were polymorphic and 48 markers among these 100 loci were amplified. Such a choice was made because of their proximity to genome areas in which QTLs related to soybean seed oil and protein contents were previously reported. Therefore, nine linkage groups were generated covering genomic segments of A1, B1, D1a, G, I, M, and O groups. Sixteen markers had significant association, explaining 3.1 to 25.4% phenotypic value, while 13 markers were also linked to the protein content. This result reflects the likelihood that common regions simultaneously act to control two distinct traits. Thus, the highest R^2 values for both the protein and oil content were observed for the same marker, Satt239, from LG I indicating that this region greatly affected the QTL for both the characteristics. These values are in agreement to those obtained in the present study.

Shi et al. (2010), working with 150 soybean genotypes, evaluated 65 microsatellite

markers to map oil and protein contents. Of those, 13 were found to be associated with the oil content and 19 with the protein content and these were distributed among 11 and 14 linkage groups, respectively, with 12 linked to both the parameters, simultaneously.

It was observed that the QTLs for oil and protein contents in the two groups of soybean cultivars and lines were distinct for the different microsatellite markers and environments. Overall, microsatellite markers specific for both the oil and protein levels were found, signifying a pleiotropic effect on both the parameters. With respect to the oil content, 21 associations were observed in group I. In this group, 21 associations were also observed for the protein content. However, in group II, 41 associations were observed for the oil content whereas 38 were found for the protein content. Generally, 13 markers were associated with both the characteristics; Sat239, Satt384, and Satt562 were significant for the assisted selection for the oil and protein content, whereas Satt310 was specific for the selection of oil content and Satt567 for the protein content as assessed by their respective magnitudes under different growth environments (Rodrigues et al., 2013).

Comparing the current genetic map with the others obtained from bi-parental crossings of elite lineage and cultivars, it is noteworthy that the Lineage 69 x Tucunaré cultivar map was biased, necessitating the need to include new markers. Population derived from this cross demonstrated sufficient genetic variability both for seed oil content and for polymorphism at the molecular marker level. Therefore, such information warrants further studies for QTL detection and mapping for the same trait to validate the results of this study.

In conclusion, major and minor QTLs were found for soybean oil content in the mapped population. QTLs not mentioned in previous studies were mapped on linkage group K, which is considered less significant (Sat_044 - Satt499), but higher in linkage group H (Satt353 - Satt629). These QTLs may contribute to saturate the mapping of seed oil content, in addition to being used for assisted-selection in breeding programs.

Conflicts of interest

The authors declare no conflicts of interest.

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