

## ASSOCIATION ANALYSIS USING SSR AND SNP LOCI TO FIND QTL FOR SEED OIL CONTENT IN SOYBEAN

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Brazil is known by its great variability in seed yields that may be used to extract vegetable oil. Nowadays, soybean is the best alternative among all oilseed crops, because of the large cultivated area throughout the country, the adequate levels of productivity and quality of oil (viscosity and cetane ratio), the short-term cycle (nearly four months from seeding to harvesting) and the network of crushing industries available nationwide. The objective of this research was to identify molecular markers associated with QTL regions related soybean oil content and composition of its main fatty acids.

Soybean accessions from different regions of the world were evaluated in field conditions of ESALQ/USP for years 2010 and 2011. Oil and oleic content of seeds were estimated by gas-chromatography. Genomic DNA samples were prepared from a lyophilized bulk of leaf tissue of each accession and polymorphism of SSR loci (Simple Sequence Repeat) and some genes (SNP loci- Single Nucleotide Polymorphism) involved in oleic biosynthesis were detected in DNA Analyzer.

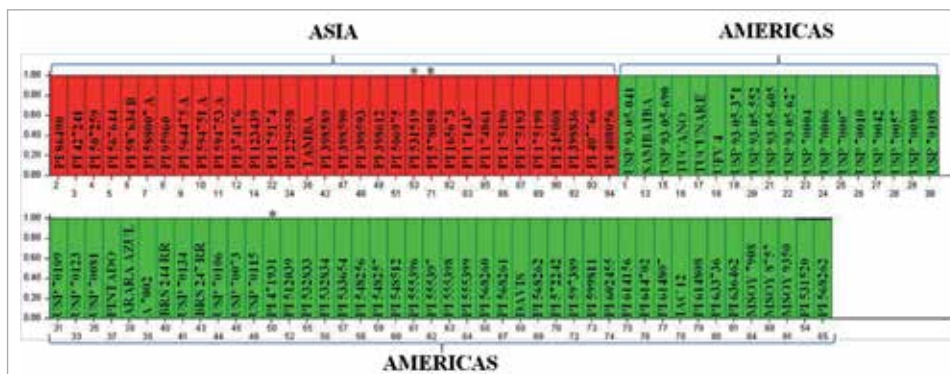


Figure 1. Two germplasm clusters, red and green, based on Bayesian analysis for the 94 soybean accessions analyzed by using 142 SSR markers. Details of identification of accessions and their geographical origins are indicated. \* Marks refer to the accessions present in a cluster that not correspond to their origins

Accession	Palmitic	Oleic	Linoleic	Linolenic	Satt294	Satt386	Satt586	Satt571	Satt270	Sat_224	Sat_144	Satt487			
					268	286	184	217	199	220	149	179	240	243	134
PI 568261	5.61	50.96	34.08	4.07		+	+								
PI 531520	8.65	54.05	28.80	2.41		+						+			
PI 578058	9.57	45.95	36.51	2.47		+		+	+			+			
PI 599811	3.24	42.73	43.76		+			+				+			
PI 568260	5.40	56.18	28.16			+	+					+			
PI 512039	9.37		44.00					+				+			
USP 70109	9.09			4.80		+		+	+			+			
USP 70106	9.19			4.31		+		+	+			+			
PI 602455	3.32					+		+				+			
PI 532833	7.41					+						+			
PI 614702		38.82	42.04	2.96			+		+			+			
PI 533654		38.26	38.93	4.06	+		+					+			
PI 555396		37.64	40.73			+		+	+			+			
USP 70081		37.35	42.57			+		+	+			+			
PI 633736		35.30		4.17	+		+		+			+			
PI 597389				4.50	+		+	+	+	+		+			
USP 70108				4.80	+		+	+	+	+	+	+			

Figure 2. Molecular profiles and fatty acid content soybean accessions based on the best alleles from each of the 8 SSR loci that were significantly associated with four fatty acids. (+) Symbol indicates presence of allele

## SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Based on polymorphisms at SSR loci, the soybean accessions were divided into two clusters corresponding to accessions from Asian and American gene pools (*Figure 1*). Linkage disequilibrium (LD) among the SSR loci suggested a structure across the soybean genome (LD decay) of approximately 12 cM. Pairwise kinship estimates among the accessions showed low values. Association analysis was performed by a mixed linear model (MLM) that identified 110 significant associated marker-traits. Oleic acid content had the highest number of associated SSR loci (33), followed by linoleic acid (26), palmitic acid (24), oil (16) and linolenic acid (14).

Modifying the fatty acid content of soybean oil for improved functionality is the objective of many soybean breeders. Increasing oleic acid levels and decreasing linoleic and linolenic acid levels makes soybean oil healthier for human consumption. Moreover, to optimize the fuel characteristics of soybean oil for use in biodiesel, it has been suggested that oils that are high in oleic acid and low in palmitic acid should be developed. We identified specific alleles at eight loci that yielded high performing soybeans with respect to balance of fatty acid content and these are presented in a crossing matrix (*Figure 2*).

We also used to candidate gene approach for association analysis. We observed single nucleotide polymorphisms (SNP) in genes FAD2-1 and FAD2-1B after sequencing of 800 bp of 20 soybean accessions that presented high and low oleic acids content. The sequences were highly similar to sequences present in gene bank (96 and 95%, to FAD2-1 and FAD-1B gene, respectively) but they had not any polymorphism significantly linked to fatty acids.

In conclusion, our analyses of SSR loci and fatty acid content were consistent with previous findings and also identified many new markers, which may be useful for quickly improving the fatty acid composition of soybean oil. By using the favorable individuals identified in this study, it may be possible to exploit the soybean germplasm using the suggested SSR loci and allele matrix to allow breeders to strategically plan fatty acid breeding programs.

## MAIN PUBLICATIONS

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