

Genetic diversity in Brazilian soybean germplasm

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Abstract: Genetic diversity is an essential factor for the success of any plant breeding program and should be considered to ensure genetic gain through breeding. In Brazil, research on the genetic diversity and population structure of soybean is required since the species is an important commodity of the country. The study addressed the genetic diversity and population structure of 77 soybean genotypes using 35 SSR markers. The estimate of the diversity index showed that the level of genetic diversity in the soybean collection is low. Similarly, the Jaccard coefficient and Bayesian model based on clustering analysis confirmed the low diversity among soybean genotypes, providing evidence for the assumption of a genetic bottleneck effect on Brazilian soybean genotypes. The results also reinforced the importance of finding and incorporating new genetic resources of soybean in the genetic pool of Brazilian soybean to warrant genetic gain in soybean breeding in the future.

Key words: *Glycine max*, molecular markers, SSR, plant breeding.

INTRODUCTION

The expansion of soybean (*Glycine max* [L.] Merr.) around the world is remarkable. Over the past few decades, the soybean acreage increased drastically, making this crop one of the most widely cultivated worldwide (Phalan et al. 2013), mainly due to its outstanding role as protein and oil source (Clemente and Cahoon 2009). In Brazil, the increase in cultivated area was closely associated with management innovation. As a result, yield rates increased in most cultivated areas (Ray et al. 2012), and the country became the second largest producer in the world (USDA 2017).

With regard to innovation, the development of new crop management technologies and the release of better-adapted genotypes can be considered key factors for the establishment of soybean as one of the major crops in Brazil (Sedyama et al. 2012). Generally, plant breeding has contributed markedly to gains in crop yields, including of soybean. In Iowa for example, plant breeding obtained a genetic gain of 79% between 1930 and 2011 (Smith et al. 2014). However, the success of breeding programs in any crop species depends on the availability of a genetic pool with adequate diversity and its management.

A gradual reduction of genetic diversity would lead inevitably to a reduction in the potential of genetic gains, increasing the susceptibility to biotic stress

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and reducing the adaptability to environmental changes (Smith et al. 2015). Therefore, breeders should focus on the enrichment of genetic diversity in the breeding population, to ensure continuous increases in genetic gain in the long run.

The final goal of genetic diversity studies is the understanding of variation among genotypes or groups of genotypes (Mohammadi and Prasanna 2003). To this end, all types of data can be used, e.g., of genealogy, morphologic traits, the biochemical profile, genotypic data, and many others. Commonly, SSR markers are used in genetic studies, due to their multiallelic nature and repeatability in laboratories (Kaga et al. 2012, Rodrigues et al. 2008). The characterization of plant genotypes by PCR-based DNA markers provides reliable data, for being free of the influence of environmental factors, unlike other methods.

In view of the importance of deepening the understanding on the genetic diversity and population structure of soybean for breeding programs, this study assessed the genetic diversity and population structure of 77 soybean cultivars representative of the genetic resource in Brazil and routinely used in agriculture and research.

MATERIAL AND METHODS

Seventy-seven soybean genotypes, which represent different maturity groups released in Brazil and are part of the breeding program of the Federal University of Lavras (UFLA) were included in this study. The list of the 77 soybean genotypes and their description is shown in Table 1.

The study was carried out in the Laboratory of Molecular Genetics of the Department of Biology (DBI), UFLA. The DNA extraction was performed as described by Pereira et al. (2007). After DNA samples of each genotype were extracted, the genomic DNA was quantified using a *NanoVue GE* spectrophotometer and standardized to 30 ng μL^{-1} for SSR genotyping. A total of 35 SSR primers were used in this study. The SSR genotyping was realized using the PCR reaction mixture composed of 5.7 μL Milli-Q water, 3 μL 5X buffer (Green GoTaq[®] Flexi Buffer - Promega), 2 μL MgCl_2 (Promega), 100 μM of each deoxyribonucleotide (dATP, dGTP, dCTP, and dTTP), 0.3 μL Taq (5 U μL^{-1}), and 3 μL of each primer pair.

Amplifications were performed in Eppendorf Mastercycler[®] Thermal Cyclers, using the following program: an initial denaturation step at 95 °C for 2 min, followed by 32 cycles at 94 °C for 30 sec each for DNA denaturation, 50 sec for primer annealing at 55 °C, 40 sec for extension at 72 °C, followed by a final extension at 72 °C for 4 min.

The reaction products were maintained at 4°C and electrophoresed on polyacrylamide gel 6% in TBE buffer (0.045 M Tris- borate and 0.001 M EDTA), at 240V for 1 h 15 min. The gel was immersed in a silver nitrate solution (0.2% AgNO_3) for 10 min, washed with water and slowly stirred in a NaOH solution (3% NaOH, 0.5% formaldehyde) until clear visualization, and photographed with a digital camera.

The DNA fragments were codified as presence/absence to form the allelic matrix for each SSR primer.

Data analyses

To study the genetic diversity and population structure, the soybean genotypes were clustered according to their maturity group and variety development method (transgenic or conventional). The relative maturity group (RMG) was determined according to the macroregional classification in the state of Minas Gerais, Brazil (Macro Region 3) where: RMG I (RMG < 7.6); RMG II (7.6 < RMG < 8.2) and RMG III (RMG > 8.2).

The genetic distance of Slatkin (Slatkin 1995) among the soybean genotypes was estimated using software PowerMarker V3.25 (Liu and Muse 2005). Furthermore, the Nei diversity index (Nei 1973), Shannon-Weaver index and percentage of polymorphic loci (P%) were estimated with software Popgene V1.32 (Yeh and Boyle 1997). The Jaccard similarity coefficient was estimated using R statistical environment (R Development Core Team 2014), and used to plot a UPGMA dendrogram with software Mega (Tamura et al. 2007). Analysis of molecular variance (AMOVA) was performed using software GenAlEx 6.501 (Peakall and Smouse 2006).

The Bayesian model based population structure was analyzed using software Structure 2.3.4 (Pritchard et al. 2000) with 10000 burn-ins and 100000 MCMC iterations. To determine the appropriate number of clusters, the process was performed from K = 1 to 12, with 21 interactions for each K. Finally, the ideal cluster number (k), or true k, was determined by the DK statistics K (Evanno et al. 2005), using online software Structure Harvester (Earl and von Holdt 2012).

Table 1. Name, source, maturity group, type and year of release of the evaluated soybean genotypes in this study, *RMG – Relative Maturity Group

#	Genotypes	Source	Type	Year of release	RMG*
1	SUPREMA	Nidera	Conventional	1998	III
2	TMG 801	FMT / TMG / UNISOJA	Conventional	2008	II
3	BRSMG 820 RR	Embrapa / Epamig	Transgenic	2013	II
4	BRSMG 250 (NOBREZA)	Embrapa	Conventional	2005	II
5	MG/BR 46 (CONQUISTA)	Embrapa / Epamig	Conventional	1998	II
6	NA 7337 RR	Nidera	Transgenic	2008	I
7	BRSGO 204 (GOIANIA)	Embrapa / AGDRF	Conventional	2009	II
8	BRSGO JATAÍ	Embrapa / EmaterGO	Conventional	1998	III
9	BRSMG 68 (Vencedora)	Embrapa / Epamig	Conventional	1998	II
10	SYN 1059 (V-TOP)	Syngenta	Transgenic	2010	I
11	TMG 123 RR	FMT / TMG / UNISOJA	Transgenic	2007	I
12	L8307 RR	Monsoy	Transgenic	2006	III
13	BRSMG GARANTIA	Embrapa / Epamig / APSEMG / COPAMIL	Conventional	2000	III
14	P 98Y11	Pioneer	Transgenic	2006	II
15	NK 7074 RR	Syngenta	Transgenic	2007	I
16	BRS SILVANIA RR	Embrapa	Transgenic	2003	III
17	M-SOY 6101	Monsoy / CTPA	Conventional	1998	I
18	ELITE	COOPADAP	Conventional	2001	III
19	M7211 RR	Monsoy	Transgenic	2007	I
20	BRSMG 850 GRR	Embrapa	Transgenic	2007	II
21	MONARCA	COOPADAP	Conventional	1999	III
22	BRSGO LUZIANIA RR	Embrapa	Transgenic	2007	III
23	TMG 1181 RR	FMT / TMG / UNISOJA	Transgenic	2009	II
24	P 98Y30	Pioneer	Transgenic	2009	III
25	M-SOY 8001	Monsoy	Conventional	1998	II
26	BRSMG 772	Embrapa / Epamig	Conventional	2013	II
27	BRS VALIOSA RR	Embrapa	Transgenic	2003	II
28	NS 5106	Nidera	Transgenic	2012	I
29	NS 5151 IPRO	Nidera	Transgenic	2012	I
30	NA 5909 RG	Nidera	Transgenic	2008	I
31	5D 615 RR	TMG	Transgenic	2011	I
32	CD 215 RR	COODETEC	Transgenic	2011	I
33	BMX POTENCIA	Brasmax	Transgenic	2007	I
34	M-SOY 7908	Monsoy	Transgenic	2004	II
35	TMG 7161	TMG	Transgenic	2010	I
36	TMG 1176	FMT / TMG / UNISOJA	Transgenic	2009	II
37	BRS MG 760 SRR	Embrapa / Epamig	Transgenic	2010	II
38	TMG 132 RR	TMG	Transgenic	2008	III
39	5D 688 RR	COODETEC	Transgenic	2010	I
40	BRS 137	Embrapa	Conventional	2001	I
41	BRSMG 752 S	Embrapa	Conventional	2008	I
42	BRSMG 800 A	Embrapa / Epamig	Conventional	2010	II
43	CD 238 RR	COODETEC	Transgenic	2010	I
44	CD 250 RR	COODETEC	Transgenic	2010	I
45	CD 237	COODETEC	Transgenic	2009	II
46	BRS 284	Embrapa	Conventional	2007	I
47	BRSMG 790A	Embrapa / Epamig	Conventional	2009	II
48	BRSMG 780 RR	Embrapa / Epamig	Transgenic	2011	II
49	NA 7255 RR	Nidera	Transgenic	2008	I
50	NS 7100	Nidera	Transgenic	2009	I

51	CD 202 RR	COODETEC	Transgenic	2011	I
52	NS 7114	Nidera	Transgenic	2012	I
53	5D 711 RR	COODETEC	Transgenic	2010	I
54	NK 7059 (V-MAX RR)	Syngenta	Transgenic	2007	I
55	EMGOPA 316	EMATER-GO	Transgenic	2007	II
56	IAC 19	IAC	Conventional	1998	I
57	TMG 1174 RR	FMT / TMG / UNISOJA	Transgenic	2011	I
58	TMG 1179 RR	FMT / TMG / UNISOJA	Transgenic	2009	II
59	TMG 401	FMT / TMG / UNISOJA	Conventional	2007	II
60	BRSMG 810 C	Embrapa / Epamig	Conventional	2007	II
61	TMG 7262	TMG	Transgenic	2011	I
62	BRS 255 RR	Embrapa	Transgenic	2005	I
63	M 9144 RR	Monsoy	Transgenic	2004	III
64	DM 118	Pioneer	Conventional	1998	II
65	5D 690 RR	COODETEC	Transgenic	2010	I
66	5G 770 RR	COODETEC	Transgenic	2010	II
67	BRS FAVORITA RR	Embrapa	Transgenic	2005	II
68	BMX FORÇA RR	GDM	Transgenic	2008	I
69	NS 7200	Nidera	Transgenic	2011	I
70	TMG 127 RR	UNISOJA / FMT	Transgenic	2007	I
71	MONSOY 8000 RR	Monsoy	Transgenic	2004	II
72	CAC 1	COOPADAP	Conventional	1998	I
73	CD 2737 RR	COODETEC	Transgenic	2012	I
74	CD 2630 RR	COODETEC	Transgenic	2011	I
75	BRSMG 750 SRR	Embrapa / Epamig	Transgenic	2007	I
76	NA 7620 RR	Nidera	Transgenic	2008	II
77	ANTA 82	FMT / TMG / UNISOJA	Transgenic	2008	I

RESULTS AND DISCUSSION

Of the analyzed primers, the lowest value for both genetic diversity indices was found for primer Satt222, and the highest Nei's genetic diversity index for primer Satt251 (Table 2). Primer Satt338 reached the highest Shannon index. The mean values for the genetic diversity indices were 0.3863 for Nei's index and 0.6294 for the Shannon index. The highest number of polymorphic alleles was observed at locus Satt270. However, 21 loci had only 2 polymorphic alleles (Table 2).

The polymorphic information content (PIC) was determined for each marker (Table 2), and the mean value was 0.3259. The highest PIC (0.6137) was observed for primer Satt338 and the lowest for Satt222 (0.0253).

A comparison of the soybean varieties obtained by transgenic and conventional breeding indicated slightly higher genetic diversity indices for the varieties derived from conventional breeding (Table 2). The transgenic cultivars had 100% and the conventional group 88.57% polymorphic loci.

Except for RMG III, the number of alleles was similar for all subsets. Subset RMG II had the highest and the RMG I the lowest index for both genetic diversity indices. Moreover, these two contained the highest PIC. For subset RMG III, the smallest number of polymorphic alleles and lowest Shannon diversity index were found (Table 3).

For the transgenic and conventional subsets, AMOVA showed that 99% of the total variation was partitioned within the subsets and the remaining 1% among subsets. Similarly, for the maturity groups, 94% of the total variation was partitioned within and 1% among the groups (Table 3).

The Bayesian model-based clustering analysis clustered the 77 genotypes in two principal groups (K=2). The first group (red) consisted of mostly short-season or early cultivars, while the second group (green) of mostly long-season or late cultivars. There is certain degree of admixture represented mostly by genotypes with medium cycle, but also by RMG I and III (Figure 1). The formation of only two groups indicated low genetic diversity among the assessed soybean genotypes.

Low genetic diversity was observed among the soybean genotypes and in all subsets analyzed in this study. The genetic diversity indices estimated in this study can be considered low when compared to results previously described for this species. In 100 vegetable soybean cultivars, Dong et al. (2014) analyzed the genetic diversity and reported a mean Nei index of 0.6286. In an analysis of the genetic diversity of 10 landrace populations in China, Wang et al. (2014) found a Shannon-Weaver index of 2.038. The high Shannon-Weaver indices observed by the authors in these two studies may be explained by the kind of genotypes used (vegetable and landrace). The selection pressure on vegetable soybean was lower than on the grain-type. Moreover, landrace genotypes are closer relatives of the wild genotypes (*G. soja*), which naturally have a high level of genetic diversity. Most genotypes included in this study are improved soybean genotypes subjected to a rather high selection pressure.

Low diversity in a germplasm is particularly alarming when we consider the vulnerability of agriculture under the impact of changes in climate patterns. The development of genotypes with better performance at high temperatures, high CO₂ concentration, and under water and salt stress has become a challenge that must be met with a certain urgency due to the ongoing climate changes (Ceccarelli et al. 2010). However, facing these challenges and developing soybean cultivars with the desirable traits requires a diversification of the genetic background of the current breeding population by incorporating new genetic backgrounds from other countries.

The genetic base of Brazilian soybean germplasm is known to be narrow (Wysmierski and Vello 2013, Miranda et al. 2001). The frequent use of a small set of genotypes in the breeding process could be considered a key factor for the loss of genetic diversity. Most of the Brazilian soybean germplasm is derived from four genotypes (CNS, S-100, Roanoke and Tokyo), which contribute with more than half of the genetic base to the cultivars released in Brazil.

Table 2. Microsatellite loci, number of alleles (n), Nei's genetic diversity index (H), Shannon-Weaver index (I) and Polymorphic Information Content (PIC)

Primer name	n	H	I	PIC
Satt200	2.0	0.4979	0.6910	0.3739
Satt239	3.0	0.4876	0.8249	0.4241
Satt270	5.0	0.5943	1.1463	0.5508
Satt196	2.0	0.1214	0.2403	0.1141
Satt492	2.0	0.1847	0.3315	0.1676
Satt358	3.0	0.3771	0.6541	0.3298
Satt012	4.0	0.5158	0.9784	0.4749
Satt076	2.0	0.4252	0.6164	0.3348
Satt225	2.0	0.4898	0.6829	0.3698
Satt137	3.0	0.4192	0.7532	0.3810
Satt475	3.0	0.3287	0.6089	0.2995
Satt241	3.0	0.4557	0.6945	0.3631
Satt345	3.0	0.6044	0.9908	0.5213
Satt426	2.0	0.2088	0.3638	0.1870
Satt251	3.0	0.6610	1.0900	0.5869
Satt509	2.0	0.2922	0.4678	0.2495
Satt338	4.0	0.6606	1.2303	0.6137
Satt369	3.0	0.4459	0.7856	0.4005
Satt342	2.0	0.4581	0.6506	0.3532
Satt274	2.0	0.2449	0.4101	0.2149
Satt520	2.0	0.4554	0.6478	0.3517
Satt052	2.0	0.4932	0.6863	0.3716
Satt222	2.0	0.0256	0.0693	0.0253
Satt302	2.0	0.4992	0.6924	0.3746
Satt146	2.0	0.4369	0.6286	0.3415
Sct_034	2.0	0.2476	0.4135	0.2169
Satt553	3.0	0.3542	0.6589	0.3259
Satt486	2.0	0.0263	0.0708	0.0260
Satt386	2.0	0.2975	0.4741	0.2533
Satt513	4.0	0.4932	0.8421	0.4163
Satt476	3.0	0.4879	0.7285	0.3810
Satt471	2.0	0.1244	0.2449	0.1167
Satt150	2.0	0.4473	0.6395	0.3473
Satt567	2.0	0.3682	0.5548	0.3004
Satt384	2.0	0.2896	0.4648	0.2477
Mean	2.5429	0.3863	0.6294	0.3259

Table 3. Evaluated soybean subsets (Subsets), number of genotypes per subset (#), mean of observed alleles (n), Shannon-Weaver diversity index (I), Nei diversity index (H), polymorphism percentage (PP)

Subsets	#	n	I	H	PP(%)	AMOVA	
						Among (%)	Within (%)
Conventional	23	2.4	0.6198	0.3817	88.57	1	99
Transgenic	54	2.4857	0.6102	0.3761	100		
RMG I	38	2.4286	0.5611	0.3426	97.14	6	94
RMG II	28	2.4571	0.6230	0.3823	97.14		
RMG III	11	2.0571	0.5532	0.3583	80		
Total	77	2.5429	0.6294	0.3863	100		

If we consider the 14 soybean genotypes used in the breeding program, their contribution to the genetic base can reach up to 92.4% (Wysmierski and Vello 2013). This conclusion is supported by the result of this study, where 77 soybean cultivars clustered into only two groups, with a high fixation index in each cluster. In summary, the soybean genetic base used in the breeding program of Brazil was constructed from a small number of genotypes. Our data confirmed this pattern and reinforced the results described by Wysmierski and Vello (2013). In addition, the methods of soybean breeding contribute to aggravate the genetic bottleneck, since backcrossing is a routinely applied method to introduce qualitative traits into an elite cultivar, particularly in the case of transgenic lines. The genetic diversity indices of the subset of transgenic cultivars in this study were lower than those of traditional genotypes, despite the higher number of genotypes included in this group (Table 3).

The development of transgenic cultivars by backcrossing can lead to a reduction in the genetic richness since a low number of recurrent parents and a few donor parents carrying the transgenic segment are used in the process. Furthermore, the small number of transformed lines reduces the range of genetic variation available for selection by the plant breeder (Sneller 2003).

Considering the rising demand for transgenic and early cultivars in the seed market, we can assume that the man-made genetic bottleneck is currently still an ongoing process that should be mitigated. The concern about genetic diversity resulting in efforts to enrich the soybean genetic pool can be helpful to preserve long-term performance increments by means of plant breeding.

The result of AMOVA showed that the absence of a clear differentiation among the groups of soybean genotypes indicates a narrow genetic base of the soybean cultivars grown in Brazil (Table 3). Therefore, soybean breeders should follow a strategy of broadening the genetic base of different groups by the incorporation of new genetic bases, particularly from landraces.

Most of the soybean cultivars analyzed in this study are early genotypes (RMG I). Nowadays, early cultivars have become rather popular among Brazilian farmers who use a production system based on crop succession, with an early-maturing cultivar in the summer followed by second-season crop after a short time gap. This widely applied system is called “safrinha” or late growing season in Brazil, and aims at a maximized land use in relation to time. Thus, this production model drives the seed companies to focus on an accelerated development of early cultivars, which may be another factor squeezing the genetic bottleneck.

The lowest Nei diversity index ($H=0.3426$) was observed in the early group (RMG I), suggesting low genetic diversity within this group. The subset RMG III obtained the lowest Shannon-Weaver index, suggesting low diversity as well. Nevertheless, RMG III is composed of a smaller number of genotypes, which can result in an underestimation of its diversity range (Table 3).

In our results, the absence of a clear clustering pattern in the dendrogram regarding relative maturity groups, and the short distance between clusters (Figure 2) suggested that the early and late cultivars may share common alleles obtained from the same parents used in the breeding process.

A pattern was observed in the population structure, where two groups were formed. The first group consisted of

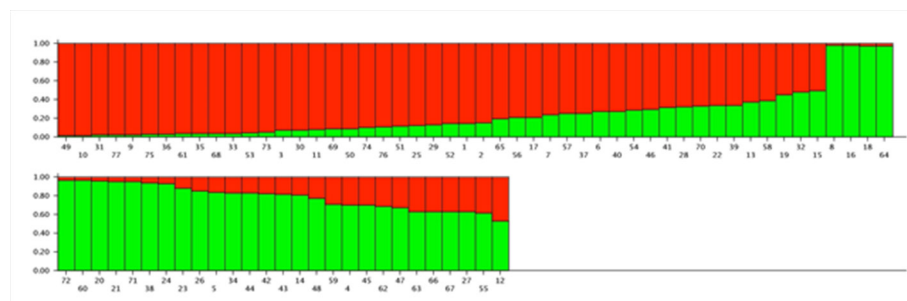


Figure 1. Two subgroups inferred from STRUCTURE analysis. The vertical coordinate indicates the membership coefficients for each plant, and the digits on the horizontal coordinate represent the reference number of the accessions listed in Table 1.

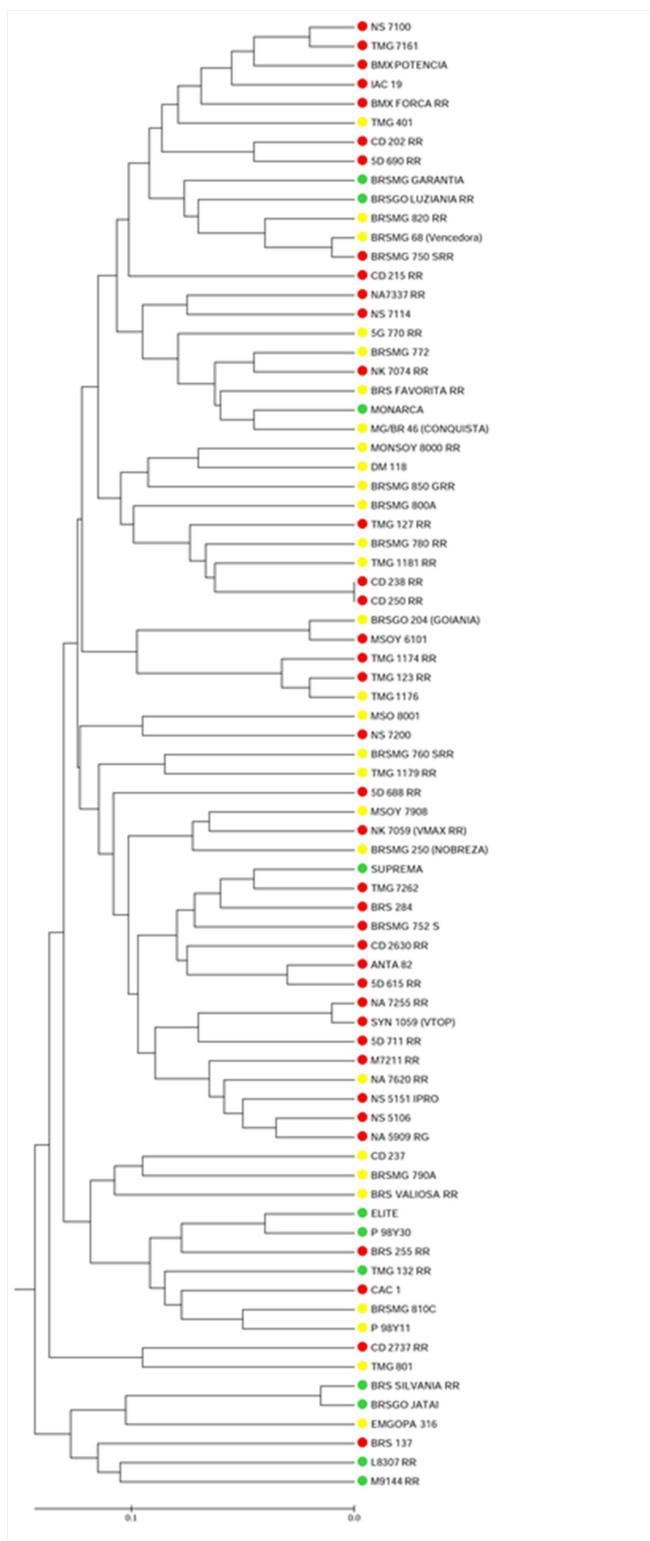


Figure 2. Dendrogram based on Jaccard's similarity coefficient, showing soybean genotypes of different maturity groups: RMG I (red), RMG II (yellow), RMG III (green).

73.9% plants from subset RMG I and the second group of 61.3% plants from subset RMG II and 25.8% from RMG III, indicating a higher allele frequency of alleles associated to early genotypes in the first group and in the second group a higher frequency of alleles associated to late genotypes (Figure 1).

In general, the result evidenced low genetic variability among soybean cultivars, reinforcing previous results. The popularization in the development and use of early transgenic cultivars should be followed by mitigating measures to alleviate the human-made bottleneck. In this context, the soybean breeding programs need to consider the genetic diversity issue as an important factor instead of focusing exclusively on yield and other agronomic traits.

In this regard, wild soybean species such as *Glycine soja* Seib. and Zucc. can be considered to increase genetic variation and used as an important source of alleles, despite their undomesticated characteristics (Akpertey et al. 2014). Interspecific crosses are commonly used in several crop species with the objective of introducing new alleles to overcome biotic and abiotic stress and increase the genetic diversity (Hausmann et al. 2004). Unfortunately, there is a lack of availability of this genetic material in the Brazilian germplasm (Carter et al. 2004).

Efforts for an international cooperation of sharing and exchanging soybean germplasm should be considered to avoid the surge of a genetic bottleneck in many countries of the world and to enrich the Brazilian soybean germplasm pool. In Brazil, attention must be paid to the threat of this bottleneck effect looming over the development of soybean breeding programs in the future.

An expansion of the soybean genetic base is fundamental to ensure the future progress of genetic gains by plant breeding. A more diverse genetic pool and germplasm set can facilitate the development of new varieties capable to overcome a hostile environment.

The analyzed soybean genotypes contain low genetic diversity, indicating the need to increase the variability in the genetic pool of the soybean breeding program of the Federal University of Lavras.

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