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Genetic divergence of roundup ready (RR) soybean cultivars estimated by phenotypic characteristics and molecular markers

Otávia Tiago Villela, Sandra Helena Unêda-Trevisoli*, Fabiana Mota Da Silva, Laerte Souza Bárbaro Junior and Antonio Orlando Di Mauro

Department of Plant Production, FCAV/UNESP-Jaboticabal- São Paulo State, Brazil.

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The aim of this study was to estimate the genetic diversity in 74 RR soybean cultivars from different Brazilian breeding programs. Analyzes were based on multivariate statistical techniques from phenotypic characteristics and microsatellite molecular markers (SSR). Ten agronomic traits were used in the analysis of the Euclidean distance, Tocher's clustering, UPGMA clustering and principal component analysis. Eighty-six of 100 SSR primer-pairs studied were selected based on their polymorphism information content, and analyzed using Jaccard Coefficient and UPGMA clustering method. The cultivars were clustered into seven groups according to the UPGMA and Tocher's methods, based on agronomic traits, while molecular analysis identified six groups. The phenotypic distances ranged from 0.46 to 9.79 and the dissimilarity measurements, based on SSR molecular markers, ranged from 0.07 to 0.73. Both results from agronomic traits and molecular markers showed that there is genetic variability among the RR cultivars and that the Monsanto breeding program has the most divergent germplasm. The analyzed agronomic traits and the chosen SSR markers were effective in assessing the genetic diversity among genotypes, besides proving to be useful for characterizing genetic variability of soybean germplasm.

Key words: *Glycine max*, genetic variability, phenotypic characteristic, SSR markers.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the most important commodities grown and commercialized in the world, and Brazil is currently the second largest producer with 90% of its area (24.3 million hectares) planted with GMO soybean cultivars (James, 2013). With the introduction of GMO soybean resistant to Roundup

*Corresponding author. E-mail: strevisoli@fcav.unesp.br. Tel: (55) 16- 3209-2666.

Abbreviations: CTAB, Cetyl trimethylammonium bromide; **GMO,** genetically modified organism; **PCR,** polymerase chain reaction; **SSR,** simple sequence repeat; **UPGMA,** unweighted pair group method with arithmetic mean; **RR soybeans,** roundup ready soybeans.

Ready herbicide, various public and private seed breeding companies have incorporated the RR gene into their best lines (Green, 2009). Despite the high number, the Brazilian soybean cultivars are extremely uniforms because they originated from only a few ancestral lines, which resulted in a narrow genetic base of germplasm (Miranda et al., 2007; Priolli et al., 2010; Wysmierski and Vello, 2013). This fact, together with the lack of genetic variability, brings risks for cultivars productivity levels and susceptibility to pests, pathogens and environmental stresses. The use of single resistance genes in a monoculture is a source of strong selective pressure for pathogen races capable of overcoming the resistance and also can influence the maintenance of cultivars to cope with multiple environmental stresses and changing conditions (Hajjar et al., 2008). Considering the importance of GMO soybeans for Brazilian production and the need to develop new more productive genotypes adapted to different environments, the study of the genetic diversity of RR cultivars is very important for knowing the existing variability among them and also within the breeding programs which produced them. Estimates of genetic divergence through multivariate analysis of both agronomic traits and molecular markers can supply information on the genetic variability of germplasm of various crops (Jose et al., 2009; Schuster et al., 2009; Liu et al., 2011). Multivariate techniques, such as discriminatory analysis, principal components, coordinate and cluster analysis, may be used to study genetic diversity and they have been very useful for unifying information from a series of variables related to genetic breeding.

The study of genetic diversity based on agronomic traits, mainly the quantitative ones, is indispensable considering their economic importance and the need to select superior parents. Multivariate analysis based on phenotypic data has been used to access genetic diversity of soybean (Mikel et al., 2010; Salimi et al., 2012; Peluzio et al., 2012); has also occurred with many of plants species, such as bean (Chiorato et al., 2007), cotton (Li et al., 2008) and rubber tree (Gouvêa et al., 2010).

More recently, with molecular marker technology, it has been possible to access species genotype and detect genetic variations at the DNA, which are inherited genetically. The microsatellite markers or Simple Sequence Repeats (SSR) are widely distributed throughout genomes and, can be highly polymorphic, for this reason have been successfully used to infer about genetics, phylogeny, pedigree, and identity of traits and germplasm accessions. The high level of polymorphism detected increases the resolution of the study of genealogy and genetic diversity and reduces the number of markers required to distinguish genotypes. SSR markers have been used to analyze genetic diversity in several species, including maize (Laborda et al., 2005), wheat (Huang et al., 2007) and soybean (Yamanaka et al., 2007; Mulato et al., 2010; Singh et al., 2010).

The objective of this study was to evaluate the genetic divergence among RR soybean cultivars from different breeding programs, using phenotypic data and SSR molecular markers.

MATERIALS AND METHODS

A group of 74 OGM soybean cultivars were selected to represent distinct geographical regions of Brazil. The cultivars chosen belong to public and private soybean breeding companies, which develop and commercialize the Roundup Ready technology in the country. The cultivars were numbered from 1 to 74 (Table 1), corresponding to the identification of the genotype throughout the work.

Analysis of phenotypic data

The field experiment was set up in the crop year 2011/2012 at the Fazenda de Ensino, Pesquisa e Extensão da Faculdade de Ciências Agrárias e Veterinárias (FCAV-UNESP), in Jaboticabal, São Paulo State, Brazil. The experimental area was homogeneous and each block consisted of a single 5 m row, with 0.5 m spacing between rows. This design was adopted owing to the small number of available seeds and according to the methodology used by germplasm banks, where the genotypes are planted in single rows, without repetitions (Carvalho et al., 2003; Chiorato et al., 2007). The agronomic traits were evaluated using the mean data of six plants collected at random within the block. Average values of ten agronomic traits, each based on six replicates, were subjected to multivariate analysis. They comprised: 1) number of days to flowering, 2) number of days to maturity, 3) first pod insertion height, 4) plant height at maturity, 5) lodging, 6) agronomic value, 7) number of branches, 8) number of pods, 9) weight of 100 seeds and, 10) grain productivity. The Euclidean distance was used to calculate the genetic distance among cultivars. The dissimilarity matrix was analyzed using Tocher's clustering and the method of average linkage between groups, UPGMA, in an attempt to establish cultivar groups. A principal component analysis was later used to evaluate the contribution of each variable to genetic divergence. Statistical analyses were performed using the Genes software (Cruz, 2008) and UPGMA dendrogram was constructed using Statistica software (Statsoft, 2004).

Analysis of molecular data

The genomic DNA samples were extracted from young trifoliate leaf tissues using the CTAB method, as described by Ferreira and Grattapaglia (1998). One hundred SSR primer-pairs distributed along all the 20 linkage groups of soybean were selected based on the information contained in the soybean genetic map, to provide efficient coverage of the whole genome. PCR amplifications were carried out in a 25 μl final volume containing 12 ng of genomic DNA, 4 mM $MgCl_2$, PCR 1X buffer (50 mM HCl, 10 mM Tris-HCl, pH 8.0), 200 μM of DNTP mixture, 1 U Taq DNA polymerase and 10 μM of each forward and reverse primer. A specific annealing temperature (Ta) was calculated for each SSR. The thermocycling program was composed of an initial denaturation cycle of 7 min at 94°C, followed by 32 cycles of 1 min at 94°C, 1 min at the specific annealing temperature of each primer-pair and extension of 2 min at 72°C, followed by a final elongation step of 7 min at 72°C. Amplification fragments were separated by electrophoresis on 3% agarose gels, with a TBE 1X buffer (89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA, pH 8.0). Gels were stained with ethidium

Table 1. RR soybean cultivars used in phenotypic and molecular analyses and the respective breeding programs which developed them.

Breeding programs	Cultivar	Breeding programs	Cultivar
Agroeste	1. AS7307 RR	**FT Sementes**	35. FTS Jaciara RR
	2. AS8380 RR		
Brasmax	3. BMX Apolo RR		36. GB874 RR
	4. BMX Energia RR		37. M7211 RR
	5. BMX Força RR		38. M7578 RR
	6. BMX Impacto RR		39. M7639 RR
	7. BMX Magna RR		40. M7908 RR
	8. BMX Potência RR	**MONSANTO**	41. M8230 RR
	9. BMX Titan RR		42. M8336 RR
Coodetec	10. CD214 RR		43. M8360 RR
	11. CD219 RR		44. M8527 RR
	12. CD230 RR		45. M8766 RR
	13. CD242 RR		46. M9144 RR
	14. CD243 RR		47. MSOY7878 RR
Emgopa	15. EMGOPA315 RR	**Nidera**	48. A4910 RR
			49. A6411 RR
			50. NA7255 RR
			51. NA8015 RR
Embrapa	16. BRS243 RR		
	17. BRS244 RR		52. P98Y11 RR
	18. BRS246 RR		53. P98Y12 RR
	19. BRS278 RR	**Pioneer**	54. P98Y30 RR
	20. BRS279 RR		55. P98Y51 RR
	21. BRS8160 RR		56. P98Y70 RR
	22. BRS8460 RR		57. P98Y31 RR
	23. BRSMG740S RR		58. P98Y01 RR
	24. BRSMG750S RR		
	25. BRSMG760S RR	**Soy Tech Seeds**	59. STS810 RR
	26. BRSMG850G RR		60. STS820 RR
	27. BRSMG811C RR		
	28. BRS Baliza RR		61. NK7074 RR
	29. BRS Charrua RR		62. SYN9074 RR
	30. BRS Favorita RR		63. SYN9078 RR
	31. BRS Juliana RR		64. ANTA82 RR
	32. BRS Pampa RR		65. TMG103 RR
	33. BRS Silvânia RR		66. TMG106 RR
	34. BRS Valiosa RR	**Syngenta**	67. TMG108 RR
			68. TMG115 RR
			69. TMG123 RR
			70. TMG132 RR
			71. TMG1179 RR
			72. TMG1182 RR
			73. TMG4001 RR
			74. TMG7188 RR

bromide to visualize bands. Data on the presence (1) or absence (0) of SSR bands were transformed into genotypic data in order to identify loci and alleles. The polymorphic information content (PIC) value for each SSR locus was calculated using the following

Table 2. 15 pairs of the most divergent and similar cultivars estimated from the Euclidean distance.

Order	15 most divergent pairs	
	Euclidean distance	Pairs of cultivars
1º	9.79	31-49
2º	9.73	6-31
3º	9.71	4-31
4º	9.64	3-31
5º	9.38	8-31
6º	9.34	48-31
7º	9.14	14-40
8º	9.08	45-49
9º	8.93	9-31
10º	8.87	40-45
11º	8.80	45-48
12º	8.70	10-31
13º	8.66	4-45
14º	8.65	3-45
15º	8.64	9-45
	15 most similar pairs	
Order	**Euclidean distance**	**Pairs of cultivars**
1º	0.46	3-4
2º	0.84	1-37
3º	0.86	1-64
4º	1.13	21-30
5º	1.14	1-50
6º	1.23	11-52
7º	1.25	4-49
8º	1.27	3-49
9º	1.28	6-49
10º	1.31	36-56
11º	1.32	2-47
12º	1.37	53-30
13º	1.41	4-6
14º	1.43	54-65
15º	1.48	3-6

formula:

$$PIC = 1 - \sum_{j=1}^{n} P_{ij}^{2}$$

Where, P_{ij} is the frequency of the allele j on the marker i.

The similarity measurements based on the SSR markers were calculated from the Jaccard Coefficient and converted into dissimilarity through arithmetic complement ($d_{ii'}$), with: $d_{ii'} = 1 - S_{ii'}$. A genetic distance matrix was estimated using Genes software (Cruz, 2008). Cluster analyses were performed using UPGMA method with the Statistica software (Statsoft, 2004). Clustering stability was tested by the Bootstrap procedure based on 10.000 re-sampling using the BooD program (Coelho, 2002). The dissimilarity matrices from the phenotypic and molecular data were correlated using the Genes software (Cruz, 2008). Both t and Mantel tests were employed with 10.000 simulations to attribute significance values to the data.

RESULTS AND DISCUSSION

Phenotypic diversity

The genetic distance among cultivars obtained through agronomic traits ranged from 0.46 to 9.79, indicating the presence of genetic variability among soybean cultivars (Table 2). Several authors have also found genetic variability between RR Brazilian soybean cultivars for many agronomic traits (Viera et al., 2009; Santos et al., 2011; Peluzio et al., 2009). In Table 2, there are the 15 pairs of the most divergent and similar cultivars identified on the dissimilarity matrix. The maximum Euclidean distance (dii' = 9.79) was observed between the BRS Juliana and A6411, also, among the most divergent combinations found, BRS Juliana was present in the major part. The minimum distance (dii' = 0.46) was found between cultivars BMX Apolo and BMX Energia, both belonging to the same breeding program (Brasmax). Diversity within breeding programs was evaluated for those programs that had more than five cultivars (Brasmax, Coodetec, Embrapa, Monsanto, Pioneer and TMG). The maximum and minimum distances between cultivars within their respective breeding programs were identified (Table 3). The Brasmax breeding program showed the lowest distance between cultivars (0.46). It also presented the lowest distance (3.35) between cultivars when evaluating the maximum distances among programs. The genetic similarity among Brasmax cultivar may be due to the extensive use of their best lines as parents for transferring and incorporating the RR gene. The wide variation among distance measurements indicates dissimilarity between cultivars, as well as variability among them. These results agree to that verified by Liu et al. (2011) and Malik et al. (2007), when assessed diversity among soybean cultivars is using phenotypic characteristics.

According to Sneller (2003), the advent of RR cultivars has had little impact on diversity, once this technology was widely used by many programs. However, to Mikel et al. (2010), facilitating gene transferences by replacing the two-parent breeding cross by partial backcrosses the genetic diversity within breeding programs was probably compromised. The Tocher's cluster analysis, based on genetic dissimilarity measurements, separated the 74 soybean cultivars into seven groups, where three of these consisted of a single cultivar (Table 4).

The Group I contained most of the cultivars evaluated (74.3% of the total), including at least one cultivar from each breeding program. This fact shows similarity among soybean cultivars, even coming from different breeding

Table 3. Minimum and maximum distances observed between cultivars belonging to the same genetic breeding programs.

Breeding programs	Mínimum		Maximum	
	Euclidean distance	Pairs	Euclidean distance	Pairs
Brasmax	0.46	3-4	3.35	6-9
Coodetec	2.51	10-12	6.04	10-14
Embrapa	1.13	21-30	8.43	29-31
Monsanto	1.71	37-47	8.87	40-45
Pioneer	1.55	55-56	6.79	53-58
TMG	1.93	65-72	6.68	67-68

Table 4. Clustering of the 74 RR soybean cultivars according to agronomic data, using Tocher's method based on Euclidean distance.

Group	RR soybean cultivars
I	3, 4, 49, 6, 8, 7, 48, 10, 5, 9, 12, 29, 18, 64, 1, 47, 24, 50, 37, 16, 39, 11, 52, 2, 38, 13, 71, 21, 53, 30, 61, 42, 22, 55, 23, 15, 57, 63, 54, 56, 74, 43, 27, 62, 73, 17, 36, 72, 66, 69, 35, 25, 65, 51
II	34, 68, 40, 44, 70, 46, 33
III	32, 41, 28, 60, 59, 67
IV	19, 45, 58
V	20
VI	31
VII	14

programs. This similarity is probably due to the RR gene introduction into the cultivars by the companies. The RR gene was engineered into the soybean cultivar to produce line 40-3-2, which is highly tolerant of glyphosate (Sneller, 2003). This line was used as a donor parent in traditional breeding schemes to develop RR soybean cultivars, which is used by many programs and could explains the clustering of cultivars.

Group II contained 9.4% of the cultivars, Group III 8.1% and Group IV 4.0%. The BRS279, BRS Juliana and CD243 cultivars were isolated into Groups V, VI and VII respectively, indicating that these cultivars are the most divergent. The formation of these groups is of major importance for choosing parents in breeding programs, once the cultivars in more distant groups are dissimilar and may be considered promising to develop new cultivars (Peluzio et al., 2009). Considering each breeding program, the Embrapa cultivars were distributed into six groups (I, II, III, IV, V and VI), the Monsanto cultivars into four (I, II, III and IV) and the TMG cultivars into three (I, II and III). The formation of various groups indicates the existence of genetic diversity between cultivars within the breeding programs since one of the characteristics of Tocher's classification is homogeneity within and heterogeneity between groups (Cruz, 2008). Several authors have shown similar results. Within this context, Peluzio et al. (2012) and Shadakshari et al. (2011) have used the Tocher's optimization method to estimate the diversity among genotypes evaluated by agronomic traits. Reina et al. (2014), also based on agronomic traits and using Tocher method, verified 11 cultivars separated into four genetically distinct clusters.

The genetic diversity among the seven Pioneer cultivars and among the five Coodetec cultivars was smaller, with most of the cultivars, except for P99R01 and CD243, allocated in the Group I. All the Brasmax cultivars were allocated in the Group I. The lower number of cultivars evaluated from the Pioneer, Coodetec and Brasmax programs may have resulted in the smaller genetic diversity observed. However, this factor was not important when only these three programs were compared since despite the higher number of Brasmax cultivars, they were all allocated to a single group. The UPGMA clustering, which is represented by a dendrogram (Figure 1), also resulted in the formation of seven distinct groups. Group I contained 52 cultivars, which represented 72.2% of the total cultivars evaluated. The group II was formed by 3 cultivars (4%), group III contained only 2 cultivars (2.7%). Groups IV and VI were formed by 10 and 5 cultivars, representing 13.5 and 6.75%, respectively. The cultivars CD243 and BRS Juliana formed the isolated groups V and VII, respectively.

Miranda et al. (2007) studying the genetic structure of 90 elite soybean cultivars adapted to different Brazilian environments, have concluded that the UPGMA method

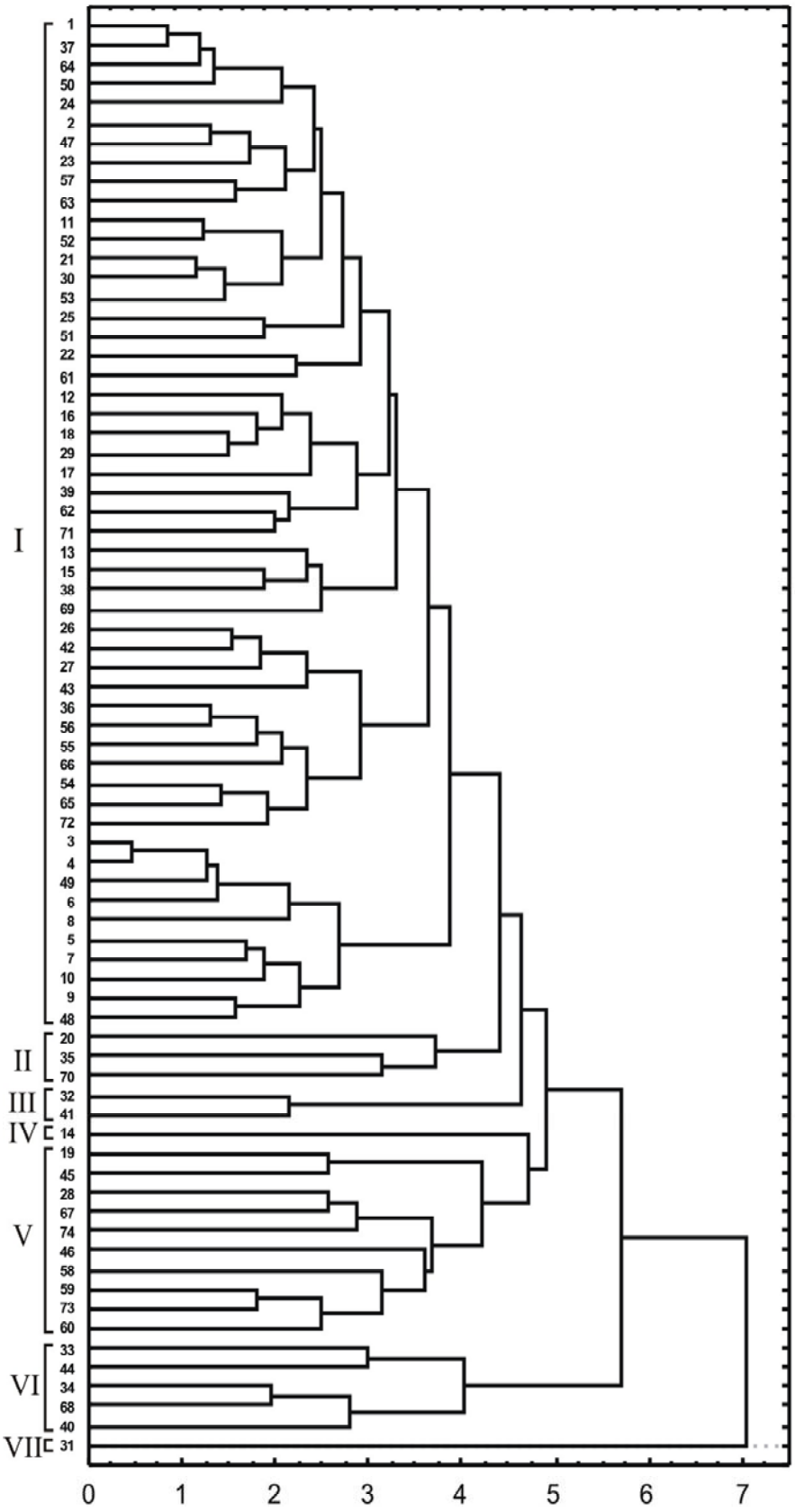

Figure 1. Dendrogram obtained by the UPGMA method, representing the genetic dissimilarity between 74 RR soybean cultivars, based on 10 agronomic traits.

was efficient for clustering the cultivars in several groups, according to their common ancestral. Moreover, this method was also efficient to demonstrate the genetic structure of the main Brazilian cultivars. The Tocher's method and UPGMA hierarchical method agreed among themselves on groups' constitution. Predominantly, the classification of genotypes between the two methods has coincided, with some exceptions such as FTS Jaciara, TMG132, TMG4001 and TMG7188 belonging to different groups and BRS 279 which was not isolated by the dendrogram analysis. In relation to diversity within the breeding programs, the only difference on groups formation was found among TMG program cultivars, which were divided into four groups (I, II, IV and VI) and not three, as was observed in the Tocher's analysis. Similarity between the clusters obtained by the Tocher's method and UPGMA hierarchical methods have also been observed by several authors studying genetic diversity in different crops, resulting in good information on the genotypes evaluated (Arshad et al., 2006; Beyene et al., 2005; Liu et al., 2011; Salimi, 2013).

Santos et al. (2011) concluded that the UPGMA and Tocher's cluster methods also agreed among them for 48 genotypes clustered into four groups. The dendrogram demonstrated the distances among genotypes, and as a result, it is possible to identify BMX Apolo and BMX Energia (both belonging to Brasmax program) as the most similar cultivars. As verified by the isolation of group VII, BRS Juliana cultivar was the most divergent and with the largest distance in the last level, when compared to 73 others. By using the dendrogram, it is possible to evaluate the groups formation and, consequently, to select genetically distinct cultivars. Studying the phenotypic diversity, Cui et al. (2001) distinguished Chinese and Americans soybean cultivars using the UPGMA methodology. Also, with the same methodology Liu et al. (2011) clustered in 5 groups, 91 cultivars belonging to Shaanxi province. By the principal components analysis it is possible to assess the genetic diversity and the influence of each characteristic for the differentiation of genotypes. The analysis of the ten agronomic traits showed that four components absorbed 80.84% of the total accumulated variation. The results of present studies are agree with those of Narjesi et al. (2007), which reported that five principal components for 30 soybean genotypes explained 80.2% variation of all data. The first principal component accounted for 36.65% of the observed variation, and the trait with the largest contribution to the diversity of cultivars was number of days to flowering. The second principal component explained 21.19% of variation and the mainly contributor was grain productivity. The third and fourth principal component absorbed 15.36 and 7.63% of the variation that were due to agronomic value and number of days to maturity, respectively.

Our result corresponded well with the study of Salimi (2013), who analyzed genetic diversity 19 soybean genotypes using agronomic traits and also showed that the number of day to flowering was the major contributor to difference cultivars. Moreover, Peluzio et al. (2009) and Shadakshari et al. (2011) also observed that number of days to flowering, grain yield and number of days to maturity were those that most contributed to differentiate genotypes.

Diversity based on molecular markers

Eighty-six of the 100 SSR markers analyzed were polymorphic and informative to evaluate the 74 cultivars (Table 5). A total of 195 alleles were identified using the polymorphic SSR primer-pairs. The number of alleles per locus ranged from 2 to 4, with a mean of 2.3. Similar results were showed by Bizari et al. (2014) when 46 soybean genotypes were evaluated, with 75 SSR primers, and found 173 alleles with a mean of 2.3 alleles per locus. Li et al. (2008), found a total of 121 alleles, generated by 35 SSR primers across 101 genotypes, and the range of allele per SSR primer was from 1 to 7 with an average of 3.45. Polymorphic information content, a reflection of allelic diversity and frequency among the soybean cultivars analyzed were generally high for all the SSR loci tested (Table 5). PIC values ranged from 0.04 (Satt 277) to 0.72 (Satt 308), with an average of 0.42. These results indicate that the selected microsatellites are very informative among the cultivars. The polymorphism of SSR loci detected in this study were in agreement with the data of Singh et al. (2010) and Tantasawat et al. (2011), who detected mean gene diversity values of 0.50 and 0.60 in a group of 44 and 25 soybean genotypes, respectively. However, these results were lower than that reported by Wang et al. (2006), who obtained PIC values ranging from 0.5 to 0.92 with a mean of 0.78, when analyzing 129 accessions of soybeans. Various authors have described the efficiency of SSR markers when analyzing genetic diversity (Fu et al., 2007; Kuroda et al., 2009; Guan et al., 2010), also were observed in the present work, whose the SSR markers selected were informative and useful for studies of genetic diversity in soybeans. Studying genetic variability in 105 soybean accessions, Shi et al. (2010) used 65 SSR primer-pairs and Mulato et al. (2010), evaluating 79 soybean accessions from different regions of the world, found a high genetic diversity among them using only 30 SSR primers.

The pairwise genetic dissimilarity between cultivars, calculated using Jaccard's similarity coefficient, varied from 0.07 to 0.73. The lowest distance was observed between BMX Força and BMX Potência (0.07) while the greatest distance occurred between BMX Titan and M7578 (0.73). The maximum and minimum dissimilarity measures found within the breeding programs of Brasmax, Coodetec, Embrapa Monsanto, Pioneer and TMG were listed in Table 6. The Brasmax breeding

Table 5. Number of polymorphic SSR primers used to evaluate 74 RR soybean cultivars, linkage group (LG), motif of repetition, chromosome number, specific temperature of each primer-pair (Ta), and number of alleles observed and values of polymorphic information content (PIC).

Number	SSR	LG	Cr	Motif	Ta (°C)	Alleles	PIC
1	SAT_001	D2	17	(AT)38	59	2	0.50
2	SAT_097	A2	8	(AT)30	52	3	0.60
3	SAT_141	G	18	(AT)11C(GA)12	49.5	2	0.44
4	SAT_250	A2	8	(AT)19	62	2	0.46
5	SATT 014	D2	17	(TTA)8	53	2	0.08
6	SATT 020	B2	14	(AAT)16	47	3	0.41
7	SATT 022	N	3	(TAT)17	58	3	0.60
8	SATT 041	D1b	2	(AAT)17	47	2	0.28
9	SATT 045	E	15	(AAT)18	44	2	0.38
10	SATT 066	B2	14	(ATT)28	48	2	0.50
11	SATT 070	B2	14	(ATT)24	47	3	0.66
12	SATT 080	N	3	(ATT)23	45.7	2	0.46
13	SATT 094	O	10	(TAT)15TG(TTA)4	47	2	0.44
14	SATT 100	C2	6	(TTA)13	47	2	0.18
15	SATT 129	D1a	1	(AAT)25	57	3	0.63
16	SATT 141	D1b	2	(ATA)25	58	3	0.59
17	SATT 154	D2	17	(TAT)7CATC(ATT)20A(CTG)4	50	2	0.49
18	SATT 166	L	19	(TTA)19	52	2	0.42
19	SATT 173	O	10	(TAT)18	52	3	0.60
20	SATT 174	A1	5	(TTA)10	55	2	0.37
21	SATT 180	C1	4	(TAT)16	42	2	0.37
22	SATT 184	D1a	1	(ATT)14(TTG)5	45	3	0.51
23	SATT 185	E	15	(TTA)29	50	3	0.61
24	SATT 187	A2	8	(TAA)18	54	2	0.45
25	SATT 193	F	13	(TAA)23	56.5	2	0.50
26	SATT 194	C1	4	(ATT)4GAGTAAATAG(TA)5	60	2	0.46
27	SATT 196	K	9	(TTA)5TTG(TTA)12(AGA)4	56	3	0.42
28	SATT 197	B1	11	(ATT)20	56.5	3	0.35
29	SATT 200	A1	5	(ATA)17	52	2	0.50
30	SATT 202	C2	6	(TTA)15	56.4	2	0.50
31	SATT 212	E	15	(TAA)9	53	2	0.20
32	SATT 220	M	7	(ATT)18ACCTTGGGA(TCC)4	55	2	0.44
33	SATT 229	L	19	(AAT)22	58	2	0.47
34	SATT 231	E	15	(TAT)32	61	2	0.26
35	SATT 236	A1	5	(ATT)19	63	2	0.50
36	SATT 238	L	19	(TTA)12	54	2	0.50
37	SATT 239	I	20	(AAT)22	61	2	0.21
38	SATT 242	K	9	(TTA)26	50	2	0.20
39	SATT 250	M	7	(TA)12	54	2	0.18
40	SATT 257	N	3	(ATA)10	60	3	0.64
41	SATT 270	I	20	(TTA)16	58.3	3	0.61
42	SATT 274	D1b	2	(TAT)18	61	2	0.21
43	SATT 277	C2	6	(ATA)41	58	2	0.04
44	SATT 286	C2	6	(ATT)18	56.8	2	0.06
45	SATT 294	C1	4	(TAT)23	60.2	2	0.50
46	SATT 302	F	12	(ATA)13AAG(TAA)4	55.9	2	0.49
47	SATT 303	G	18	(TAA)20	47.3	3	0.55
48	SATT 308	M	7	(TTA)22	62.5	4	0.72
49	SATT 313	L	19	(ATT)14	62	2	0.50

Table 5. Contd.

50	SATT 317	H	12	(TCAT)3(TTA)21	59	2	0.34
51	SATT 335	F	13	(TCT)4	57	3	0.46
52	SATT 342	D1a	1	(ATT)21	58.2	2	0.48
53	SATT 353	H	12	(TTA)17	63	2	0.50
54	SATT 355	E	15	(CAT)6(AAT)14	61	3	0.41
55	SATT 358	O	10	(ATA)19	63	3	0.46
56	SATT 371	C2	6	(TAA)11	56.2	2	0.40
57	SATT 384	E	15	(ATA)16	61	2	0.15
58	SATT 396	C1	4	(TTA)9	56	2	0.11
59	SATT 398	L	19	(ATTA)3	61	2	0.47
60	SATT 399	C1	4	(ATT)14	54.5	2	0.15
61	SATT 415	B1	11	(TAA)4	59.2	2	0.20
62	SATT 417	K	9	(AAT)18	54	2	0.49
63	SATT 420	O	10	(TAT)16	57	2	0.44
64	SATT 423	F	13	(TAT)19	50	2	0.47
65	SATT 426	B1	11	(ATT)5	62.7	2	0.13
66	SATT 434	H	12	(ATA)32	55.5	2	0.44
67	SATT 442	H	12	(TAA)35	61	2	0.40
68	SATT 449	A1	5	(TTA)21	56	3	0.63
69	SATT 458	D2	17	(TAT)30	64	2	0.50
70	SATT 468	D1a	1	(ATTT)3TGAAATTCTTCATATT(TTA)14	59	2	0.34
71	SATT 476	C1	4	(ATA)20	56.3	2	0.49
72	SATT 480	A2	8	(TAT)14	56.4	2	0.37
73	SATT 496	I	20	(ATT)13	62	2	0.50
74	SATT 510	F	13	(TAT)9	62	3	0.64
75	SATT 540	M	7	(TTA)15	58	2	0.49
76	SATT 542	D1b	2	(TAA)19	55	2	0.16
77	SATT 545	A1	5	(TTA)24	52	2	0.49
78	SATT 551	M	7	(AAT)8	54	2	0.33
79	SATT 556	B2	14	(AAT)14	54	3	0.56
80	SATT 562	I	20	(TTA)18	57	2	0.37
81	SATT 571	I	20	(ATA)14	50	3	0.52
82	SATT 591	A1	5	(ATT)17	50.8	2	0.26
83	SATT 610	G	18	(ATA)9	56.8	2	0.46
84	SATT 632	A2	8	(AAT)17	53.6	2	0.50
85	SATT 663	F	13	(TTA)27CTATTACTATTAC(TAT)4	56	2	0.21
86	SATT 703	D1b	2	(ATT)27	56.9	2	0.47
Total						195	
Mean						2.27	0.42

Table 6. Minimum and maximum measurements of dissimilarity obtained between cultivars belonging to the same genetic breeding programs.

Breeding	**Minimum**		**Maximum**	
Programs	**Jaccard's Coefficient**	**Pairs**	**Jaccard's Coefficient**	**Pairs**
Brasmax	0.0764	5-8	0.5102	4-6
Coodetec	0.2666	11-14	0.5617	10-14
Embrapa	0.1497	16-17	0.6104	16-23
Monsanto	0.3773	46-47	0.5901	39-47
Pioneer	0.1923	53-54	0.5577	54-57
TMG	0.2374	65-66	0.6031	68-73

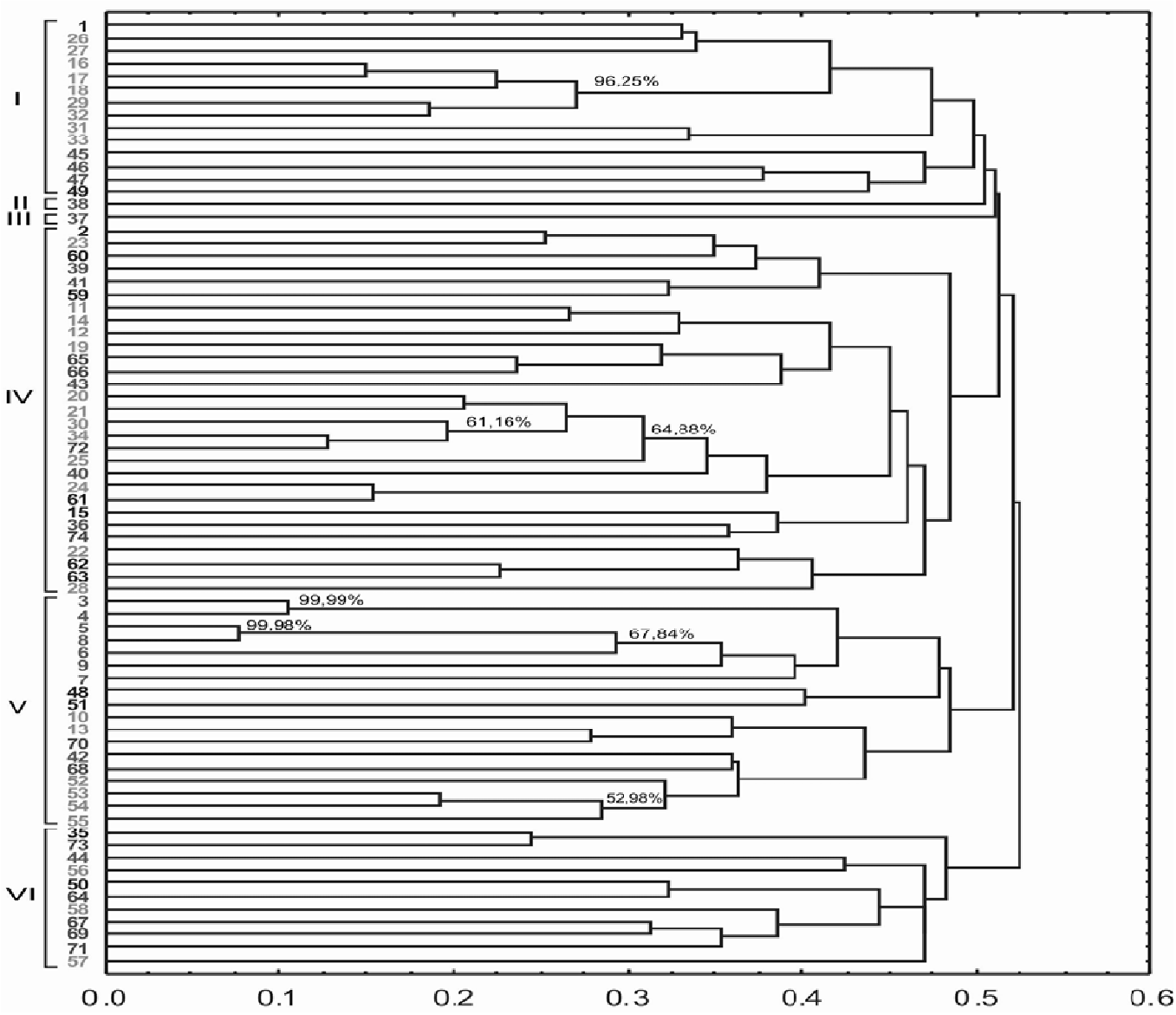

Figure 2. Dendrogram obtained using the UPGMA method, representing genetic dissimilarity among 74 RR soybean cultivars, based on 86 SSR markers. Bootstrap node support, represented in percentages, shows clustering stability.

program showed the lowest dissimilarity between cultivars (0.07), among all minimum measures observed. Moreover, it also had the lowest dissimilarity between cultivars (0.51) when compared to the maximum distances among programs, indicating the existence of lower genetic variability among their cultivars.

The UPGMA cluster analysis, based on the genetic dissimilarity matrix, showed that the 74 cultivars formed six major groups (Figure 2). Bootstrap analysis expressed high statistical support for the most part of the nodes in the dendrogram. The cophenetic correlation between the dissimilarity matrix and the dendrogram was significant at 1% of probability (0.66) by the test t. Bootstrap analysis and cophenetic correlations indicated that SSR dendrogram clustering accurately depicted estimated genetic distances among soybean cultivars. Group I contained 14 cultivars, which represented 18.9% of the total cultivars evaluated. The cultivars M7578 and M7211 formed the isolated groups II and III, respectively. Group IV was the largest group consisting of 29 genotypes (39.1%), including cultivars from almost all the breeding programs. Group V and VI were formed by contained 18 and 11 cultivars representing respectively 24.3 and 14.8% of the total genotypes evaluated. Analyzing cultivars distribution within breeding programs, the 12 genotypes from Monsanto were distributed in all the six groups formed (I, II, III, IV, V and VI) and two of them were in the isolated groups II (M7578) and III (M7211).

The 11 TMG cultivars were clustered in the groups IV, V and VI. The 19 cultivars from Embrapa showed less diversity due to their clustering in only two groups (I and IV), were the Coodetec and Pioneer cultivars, which were distributed in the groups IV and V and V and VI, respectively. All the Brasmax cultivars were clustered in the group V, indicating a greater genetic similarity between them.

Through the genealogy of some cultivars belonging to the same group, it is possible to verify parental in common, such as BRS243 and BRS244 from Embrapa. Both have genealogy Embrapa 59 and the bulk E96 246 as similar parental. The low genetic diversity found may be due to evaluation of sib lines. The dendrogram showed the formation of two subgroups exclusive to Embrapa and Brasmax breeding programs. The BRS 243, BRS 244, BRS 246, BRS Charrua and BRS Pampa cultivars from Embrapa formed a subgroup within the group I. Despite this clustering, the Embrapa cultivars were distributed into two groups, which indicated variability. The opposite occurred with the Brasmax cultivars (BMX Apolo, BMX Energia, BMX Força, BMX Potência, BMX Impacto and BMX Magna), all of them formed a subgroup within the group V, indicating a close similarity and practically no genetic variability in its germplasm. Cluster analysis using hierarchical methods have been widely used in studies of genetic diversity (Yamanaka et al., 2007; Singh et al., 2010). Wang et al. (2010) studying genetic variability in 40 soybean accessions of cultivars, landraces and wild soybeans collected from China found that wild soybeans and landraces possessed greater allelic diversity than cultivars and the UPGMA results also exhibited that wild soybean was of more abundant genetic diversity than cultivars.

Moreover, hierarchical methods have shown good agreement between the dendrograms generated and the kinship among accessions evaluated. Bonato et al. (2006) observed that the dendrogram obtained with AFLP markers was consistent with the pedigree of soybean genotypes analyzed. Priolli et al. (2010) found that the clustering of 168 soybean cultivars obtained by UPGMA method, based on the information of SSR markers, were consistent with ancestors which are common among cultivars within the same group.

Comparison between phenotypic and molecular analyses

Both the phenotypic data, represented by the agronomic traits, as the molecular data proved to be a useful tool on diversity characterization among the RR cultivars. Both methods demonstrated that Monsanto cultivars were clustered into various groups, indicating highest diversity among cultivars, which may be due to this company having been responsible for the development of RR soybeans and to their strong research effort in this area (Green, 2009). Although, the TMG cultivars have been grouped into fewer groups, they did show a relatively high genetic divergence when analyzed by both methods. Moreover, the methods equally indicated that the Coodetec, Pioneer and Brasmax programs had a low diversity since these genotypes were clustered in only one or two groups. The use of highly related genotypes as receptors of the RR gene within the soybean breeding programs may have caused the low genetic diversity observed in this study.

Li et al. (2008) observed that soybean cultivars from the same breeding programs were clustered in the same group and attributed this to a restricted use of parents in developing these cultivars. Vieira et al. (2009) also described low genetic diversity among cultivars from the same breeding program when they evaluated 53 soybean cultivars commercialized in Brazil. The formation of one group containing most of the 74 cultivars was also observed on the phenotypic and molecular analyses demonstrating the genetic similarity among RR soybean cultivars, even for cultivars belonging to distinct breeding programs. Santos et al. (2011), analyzing diversity between 48 Brazilian soybean cultivars, observed a tendency for transgenic cultivars to form a single and very similar group.

Sneller (2003), studying the genetic structure of soybean elite population in North America and the effect of recurring crosses with RR soybeans on the genetic divergence of these lines, concluded that RR technology generally had only a small impact on cultivar genetic diversity. However, based on the low diversity found between the elite lines of some companies, the author concluded that the low diversity in some programs, with the low germplasm exchange, could affect the available variability in the future. Studying the genetic structure basis of soybean in Brazil, Wysmierski and Vello, (2013) pointed out an increasing number of ancestors over all period, as well as its relative genetic contribution also increased from 46.6 to 57.6%, indicating a narrowing of the genetic base. These authors suggested if there is interest by the companies to increase the genetic base, they should choose the parents with the most divergent pedigrees. Contradicting these results, Vieira et al. (2009), Santos et al. (2011) and Peluzio et al. (2009), have detected variability among soybean elite populations in Brazil. Although both analyses shared most of the results, there were some differences. The most divergent cultivar pairs identified with Euclidean distance (phenotypic data) differed from those obtained with the Jaccard Coefficient (molecular data). However, it can be seen that for both methods, the minimum distances always occurred among the Brasmax cultivars. Differences were also observed in the cultivars clustering within the Embrapa program. Such cultivars were less divergent when analyzed by molecular markers being separated into two groups and not into six as occurred

with the phenotypic analysis.

The correlation coefficient between genetic distances estimated by phenotypic and molecular data was low but significant (r=0.11, P<0.01. t-Test and Mantel's Test with 10,000 simulations). Gouvêa et al. (2010) also observed low correlations between the genetic distances based on SSR and the phenotypic data in the rubber tree (r=0.13, P<0.01). However, Li et al. (2008) found moderate correlation coefficients (r=0.31 p < 0.01) in soybean utilizing SSR markers. Chiorato et al. (2007), on correlating matrices from agronomic variables and RAPD molecular descriptors in dry beans, also found moderate correlation coefficients (r=0.33, p<0.01). The difference between the most divergent cultivar pairs found from the Euclidean distance and Jaccard's Coefficient, as well as the low correlation between the phenotypic and molecular data, indicate that each method estimated the divergence between genotypes in a distinct way. According to Roldan-Ruiz et al. (2001), an alternative way to deal with the low correlation between genetic and phenotypic distance, would be selecting only molecular markers associated with phenotypic traits. Another factor which makes the occurrence of an association between phenotypic and molecular data more difficult to observe is that the variation detected by the molecular markers is not adaptive and, therefore, not subject to selection, in contrast to the agronomic traits, which are subject to both natural and artificial selection, as well as suffering a significant environmental influence (Vieira et al., 2005). The soybean cultivars used in this study are a representative sample of the RR cultivars grown and commercialized in Brazil. Therefore, it was possible to make an inference on the existing genetic diversity into the breeding programs that developed these cultivars. Even without the genealogical information, the dendrograms developed from the phenotypic and molecular data grouped cultivars according to their origins.

The results of this study show that some breeding programs had less genetic diversity, indicating the use of a narrow genetic base for developing their RR cultivars. The introduction of variability into soybean breeding programs to generate new combinations from the widening of the genetic base of this crop is fundamental for dealing with new demands and avoiding the risks of genetic vulnerability. The selection of more divergent cultivars, based on the dendrograms presented, is a viable alternative, which can be used commercially to avoid production losses related to the extensive use of cultivars with a narrow genetic base.

Conclusion

The existence of genetic variability between RR soybean cultivars was verified. Both agronomic traits and SSR molecular markers are useful tools for estimating the existing divergence among RR cultivars. Multivariate techniques based on agronomic traits and SSR molecular markers show differential ability to estimate genetic divergence between genotypes and should be used as complementary tools.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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