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Genetic diversity in soybean genotypes using phenotypic characters and enzymatic markers

E.V. Zambiazzi[1], A.T. Bruzi[1], A.P. Sales[1], I.M.M. Borges[1], S.R. Guilherme[2], A.M. Zuffo[3], J.G. Lima[2], F.O. Ribeiro[2], A.E.S. Mendes[1], S.H.M Godinho[1] and M.L.M. Carvalho[1]

[1]Departamento de Agricultura, Universidade Federal de Lavras, Lavras, MG, Brasil
[2]Departamento de Biologia, Universidade Federal de Lavras, Lavras, MG, Brasil
[3]Departamento de Agronomia, Universidade Estadual do Mato Grosso do Sul, Cassilândia, MS, Brasil

Corresponding author: A.M. Zuffo
E-mail: alan_zuffo@hotmail.com

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ABSTRACT. The objective of this study was to evaluate the genetic diversity of soybean cultivars by adopting phenotypic traits and enzymatic markers, the relative contribution of agronomic traits to diversity, as well as diversity between the level of technology used in soybean cultivars and genetic breeding programs in which cultivars were inserted. The experiments were conducted on the field at the Center for Scientific and Technological Development in crop-livestock production and the Electrophoresis Laboratory of Lavras Federal University. The agronomic traits adopted were grain yield, plant height, first legume insertion, plant lodging, the mass of one thousand seeds, and days for complete maturation, in which the Euclidean distance, grouped by Tocher and UPGMA criteria, was obtained. After electrophorese gels for enzymatic systems, dehydrogenase alcohol, esterase, superoxide

dismutase, and peroxidase were performed. The genetic similarity estimative was also obtained between genotypes by the Jaccard coefficient with subsequent grouping by the UPGMA method. The formation of two groups was shown using phenotypic characters in the genetic diversity study and individually discriminating the cultivar 97R73 RR. The character with the greatest contribution to the genetic divergence was grain yield with contribution higher than 90.0%. To obtain six different groups, individually discriminating the cultivars CG 8166 RR, FPS Jupiter RR, and BRS MG 780 RR, enzymatic markers were used. Cultivars carrying the RR technology presented more divergence than conventional cultivars and IPRO cultivars.

Key words: *Glycine max* (L.) Merrill; Genetic variability; Genetic dissimilarity; Plant breeding

INTRODUCTION

Soybean breeding programs stand out for the continuous search for genotypes that are resistant to pests and diseases, tolerant to water deficit, in addition to presenting high productive potential and cycles suitable for cultivation in specific environments (Vieira et al., 2009). Superior and stable genotypes represent a very critical genetic balance, which once reached additional gains, becomes more difficult to be achieved. The consequence of this balance is that most of the cultivars within a region are genetically similar, therefore, with a more limited genetic basis (Hamawaki et al., 2012; Villela et al., 2014).

Narrowing is caused by the use of similar genetic breeding programs in Brazil, and the long run may cause risks such as genetic vulnerability and reduction in levels of productivity (Wysmierski and Vello, 2013). The expansion of the genetic basis of soybean cultivars increases heterogeneity and reduces the risk of genetic vulnerability and consequently the risk of yield reduction. Situation contrary to this is likely to occur when working with high similarity between parents used at crosses for developments of new cultivars (Brondani et al., 2003).

To the breeders, it is important to obtain great genetic variability for selection in plants, imposition results that in fact promote significant genetic gains (Bernardo, 2010; Cruz et al., 2011). To obtain segregating populations, a parent's choice to be used in hybridizations is needed. Different strategies can be used to identify parents seeking for cross-breeding realization. Among the existing alternatives, the method to estimate the genetic divergence is highlighted (Torres et al., 2015).

Different markers allow understanding and uniquely studying the genetic variability, enabling planning crosses to maximize the genetic differences among genotypes, facilitating parental choice, and reducing the number of combinations to be made (Muniz, 2007). It also may contribute to enzymatic markers, by providing excellent results for breeding programs, in the search for heterotic groups promising for the constitution of hybrids in the heterosis prediction (Caixeta et al., 2009; Cruz et al., 2011).

In addition to the use of markers, the measurement of agronomic traits is also important because it allows the breeder to identify and select the best genotypes through characters of agronomic relevance, especially those of quantitative nature due to the need to succeed in the correct choice of superior hybrid combinations (Oliveira et al., 2014; Villela et al., 2014).

In parent synthesis of new population selection, it is recommended to observe whenever a set of traits of interest rather than individual traits (Val et al., 2014), as well as the association with available markers, contribute to the reliability of the results and understand the relationship between the approaches (Singh et al., 1991; Chioratto et al., 2007).

In this sense, to conduct the choice of promising parents for future hybridization, and to increase genetic variability in soybean crop, this study aimed to: i) study genetic diversity in soybean cultivars by phenotypic characters and enzymatic markers; ii) quantify the relative contribution of agronomic traits in the total variation observed; iii) evaluate the diversity between the level of technology used in soybean cultivars and breeding programs to which cultivars are inserted.

MATERIAL AND METHODS

The experiment was carried out in two stages, field and laboratory. A group of 76 soybean cultivars (Table 1) composed of 11 conventional cultivars carrying the 51 RR (Roundup Ready) and 14 cultivars carrying the IPRO technology (intact $BtRR_2$) were evaluated. The genotypes belong to public and private breeding companies that were adapted for cultivation in different regions.

Table 1. Soybean cultivars used in the genetic study diversity by phenotypic characters and enzymatic markers - UFLA, Lavras - MG, 2015.

Identification/soybean genotype		
1. 5D 615 RR	27. BRS MG 850 RR	53. M 7110 IPRO
2. 5D 6215 IPRO	28. BRS Valiosa RR	54. M 7211 RR
3. 5D 690 RR	29. CD 202 RR	55. NA 5909 RR
4. 5G 770 RR	30. CD 215 RR	56. NK 7059 RR
5. 5G 830 RR	31. CD 237 RR	57. NS 5106 IPRO
6. 95R51 RR	32. CD 238 RR	58. NS 5151 IPRO
7. 97R21 RR	33. CD 250 RR	59. NS 7100
8. 97R73 RR	34. CD 2737 RR	60. NS 7114
9. Anta 82 RR	35. CG 67 RR	61. NS 7200
10. AS 3575 IPRO	36. CG 68 RR	62. NS 7209 IPRO
11. AS 3610 IPRO	37. CG 7464 RR	63. NS 7300 IPRO
12. BMX Desafio RR	38. CG 7665 RR	64. P98Y11
13. BMX Força RR	39. CG 8166 RR	65. RK 5813 RR
14. BMX Ponta IPRO	40. FMT 0860.346/1 RR	66. RK 6813 RR
15. BMX Potência RR	41. FMT 0861.708/2 RR	67. SYN 13610 IPRO
16. BRS 213	42. FMT 0871.422/3 RR	68. TMG 1176 RR
17. BRS Favorita RR	43. FPS Antares RR	69. TMG 1179 RR
18. BRS Vencedora	44. FPS Atalanta IPRO	70. TMG 1181 RR
19. BRS MG 752 S	45. FPS Júpiter RR	71. TMG 123 RR
20. BRS MG 760 RR	46. FPS Netuno RR	72. TMG 127 RR
21. BRS MG 772	47. FPS Paranapanema RR	73. TMG 7161 RR
22. BRS MG 780 RR	48. FPS Solar IPRO	74. TMG 7262 RR
23. BRS MG 790 A	49. FPS Solimões RR	75. V Max RR
24. BRS MG 800 A	50. FPS Urano RR	76. V Top RR
25. BRS MG 810 C	51. LG 60163 IPRO	
26. BRS MG 820 RR	52. LG 60177 IPRO	

Phenotypic characters

A field experiment was conducted in the 2014/2015 season on the field at the Center for Scientific and Technological Development in the crop-livestock production of the Lavras Federal

University (UFLA) in Lavras, Minas Gerais - Brazil, (21°12'S and 44°58'W, with an altitude of 918 m). The soil is classified as a typical dystrophic Red Latosol, according to the Brazilian Soil Classification system (Embrapa, 2013). Region climate is the Cwa type with an average annual temperature of 19.3°C and normal annual rainfall of 1530 mm (Dantas et al., 2007).

The trials conducted began in November 2014 with the preparation of the experimental area, and adopting direct seeding over corn stubble with prior desiccation of the area using 960 g/ha glyphosate active ingredient. The fertilization followed the recommendations of Souza and Lobato (2004), performing in-furrow inoculations, and applying 350 kg/ha formulated N-P_2O_5-K_2O (02-30-20). The experimental plot consisted of a single row of 5 m in length, spaced 0.5 m between rows, with a design similar to that used for multiplication and regeneration of germplasm bank accessions without repetitions (Chioratto et al., 2007; Villela et al., 2014).

Seeding was carried out manually with a 15-seed density per linear meter. The inoculation was performed in the furrow after seeding, according to the recommended methodology of Embrapa (2013), with *Bradyrhizobium japonicum* bacteria at a dosage of 18 mL/kg seed - strains SEMIA 5079 and 5080, containing 10.8 x 10^6 CFU/seeds of the inoculant Nitragin Cell Tech HC® (3 x 10^9 CFU/mL). Weeds, pests, and disease controls were carried out according to the technical recommendations for the soybean crop (Embrapa, 2013). When plants were at the R_8 development stage, they were evaluated for plant height (cm): distance measured from the soil surface to the last node of the main stem, using a millimeter ruler, obtaining the average value of five random plants in the plot; first-pod insertion (cm): distance measured from the soil surface to the first pod on the main stem of the plant, using a millimeter rule, obtaining the average value of five random plants in the plot; plants lodging: estimated according to the scale proposed by Bernard et al. (1965), ranging from 1 (all erected plot plants) to 5 (above 80% lodged plot plants); full maturity: number of days counted from the emergence date of seedling until the date in which 95% pods of plants are ripened (R8 stage); one thousand seed mass: followed by recommendations from Brasil (2009), using eight replications of 100 seeds from a portion of the pure seed of each plot, where each sample was weighed individually and the results expressed in grams (g); grain yield: the yield was determined from harvest of usable area of each plot. Then, the moisture content of grains to 13% was standardized, and the yield in kg/ha was estimated. For evaluations and harvesting, only 4.0 m centers were considered, excepting 0.5 m of extremities.

For multivariate analysis performed from agronomic traits, the Euclidean distance was adopted as dissimilarity measure, chosen by not requiring experiments involving delineations with repetition. The groupings were conducted by the hierarchical method of the average link between groups (UPGMA), and also by the Tocher's optimization procedure. Construction analyses of the UPGMA dendrogram were performed with the help of the Genes program (Cruz, 2013). Dendrogram cut-off point was defined as proposed by Mojema (1977). Additionally, it has been quantified the relative contribution of agronomic traits to genetic divergence, using criteria proposed by Singh (1981).

Enzymatic markers

Initially, 12 seeds of each genotype (Table 1) were seeded in polystyrene trays with 128 cells, containing Plantmax® substrate and placed to germinate in a greenhouse with a temperature of 27° ± 2°C and air relative humidity of 80 ± 2%. When plants reached a

vegetative stage (V_2), the leaves were collected and sent to the Lavras Federal University Laboratory for electrophoresis, then, were macerated in an electric grain mill in the presence of PVP antioxidant and liquid nitrogen. For each genetic material, a sample of 100 mg ground leaves was used and added to 250 µL buffering extraction (0.2 M Tris-HCl, pH 8) 2.5 times the weight of each sample and 0.1% β-mercaptoethanol. Samples were kept at 4°C, in the presence of buffering extraction for 12 h and then centrifuged at 14,000 rpm for 30 min at 4°C. Electrophoretic race was held on 7.5% polyacrylamide gels (gel separator) and 4.5% (gel concentrator). The Gel/electrode system used was the Tris-glycine, pH 8.9, and 50 µL supernatant of samples was applied to the gel, and the electrophoresis race was performed at 150 V for 5 h. After electrophoretic gel for enzymatic systems, alcohol dehydrogenase, esterase, superoxide dismutase, and peroxidase were developed, according to Alfenas (2006), using the surface of a Transilluminator. Evaluations of the enzyme protein patterns consisted of the observation of the presence and absence of bands in each genotype designated by 1 and 0, respectively. A matrix was made of 0 and 1, and the estimate of the genetic similarity (Sg_{ij}) between each pair of genotypes was calculated considering the coefficient of Jaccard's similarity. Genotypes were grouped by the UPGMA, with the help of the Genes program (Cruz, 2013), and dendrogram cut-off point was defined as proposed by Mojema (1977).

RESULTS AND DISCUSSION

Genetic distances among cultivars obtained by phenotypic characters and estimated from the Euclidean distance (dii'), ranged from0.04 to 1.67, proving that there is genetic variability among the soybean cultivars evaluated. However, the small magnitude demonstrates low genetic variability among soybean cultivars, especially when the results are compared to those obtained by Villela et al. (2014), which estimates ranged from 0.46 to 9.79, indicating the presence of high genetic variability between accessions evaluated.

Through matrix dissimilarity, 10 pairs of the most different cultivars were identified (Table 2). The Euclidean distance (dii' = 1.67) was obtained between the pair of cultivars 97R73 RR (8) and NS 7100 (59). There was a higher frequency of pairs with larger distances when one of the components studied was cultivar 97R73 RR (8).

Table 2. Cultivar pairs (C.P.) most different and most similar estimated from the maximal (Max) and minimum (Min) Euclidean distances (dii') obtained to study genetic diversity by phenotypic characters between soybean cultivars, UFLA, Lavras - MG, 2015.

Order	(dii') Max.	C.P.	(dii') Min.	C.P.
1	1.6712	8-59	0.0450	36-53
2	1.6366	8-57	0.0813	18-28
3	1.6364	8-6	0.0928	55-65
4	1.6207	8-50	0.0946	2-10
5	1.6042	8-47	0.0996	11-14
6	1.5501	8-55	0.0999	45-48
7	1.5380	8-75	0.1074	51-2
8	1.5304	8-65	0.1076	67-11
9	1.5238	8-44	0.1100	40-73
10	1.5224	8-58	0.1252	6-57

According to Garcia (2002), heterosis level is directly related to the genetic distances between the parents; in the greater distance, there is a greater divergence between individuals.

The most similar cultivars (Table 2), which have the shortest distance, were observed between CG 68 RR (36) and (M 7119 IPRO (53), with dii' = 0.04. In spite of not being cultivars from the same breeding program, the least distance checked is probably related to a combination of cultivars coming from derived parents/guardians similar to the soybean breeding crop that has been currently based primarily on the use of cultivars already improved.

Genetic diversities within each breeding program and obtained between the level of technology employed in cultivars were also evaluated. For the diversity evaluated within each breeding programs, those which contained five or more cultivars were used, as was the case of BRASMAX Genetics (5) program; Caraíba Genetics (5); Dow AgroSciences (5); Coodetec (6); Nidera (8); Tropical Genetic Breeding (8); Pro-Sementes Foundation (8), and Embrapa (13).

Cultivars belonging to breeding program of the Pro-Sementes Foundation presented the shortest distance between cultivars (dii' = 0.09) among minimum observed (Table 3). This same program also presented the shortest distance (dii' = 0.50) when observed the maximum distances indicating less genetic diversity. Greater diversity was observed in cultivars from the TMG and Nidera program, which presented wide variation between distance measures among cultivars (dii' = 0.28 and 0.18, respectively) between minimum observed and also greater distances (dii' = 1.15 and 1.21, respectively) when observed maximum distances.

Table 3. Minimum (Min.) and maximal (Max.) Euclidean distances (dii') obtained between the level of technology employed in the genetic breeding program in soybean cultivars, UFLA, Lavras - MG, 2015.

	N	(dii') Min.	Pairs	(dii') Max.	Pairs
Technology employed					
Conventional	11	0.1601	19-21	1.1226	23-59
RR	51	0.0946	55-65	1.6364	6-8
IPRO	14	0.0928	2-10	0.9865	52-57
Breeding program					
BMX[1]	5	0.2098	12-14	0.7851	14-75
CG[2]	5	0.2044	35-37	0.5999	36-39
CD[3]	6	0.1980	30-33	0.9130	32-33
DA[4]	5	0.2923	4-5	1.0869	1-5
BRS[5]	13	0.1601	19-21	1.0633	16-27
FPS[6]	8	0.0999	45-48	0.5066	44-46
NS[7]	8	0.1810	57-58	1.2112	59-62
TMG[8]	8	0.2816	68-70	1.1563	69-72

N = number of cultivars; [1]BRASMAX Genética; [2]Caraíba Genética; [3]Coodetec; [4]Dow AgroSciences; [5]Embrapa; [6]Fundação Pro-Sementes; [7]Nidera; [8]Tropical Melhoramento Genético.

As reported by some authors (Almeida et al., 2011; Rigon et al., 2012; Villela et al., 2014) in studies evaluating diversity among soybean cultivars, a wide variation between distance measurements demonstrates the existence of divergence among cultivars, as well as the presence of variability between them; this fact was observed in this study meeting accordingly to reports of Bonato et al. (2006), who found genetic heterogeneity between and inside Brazilian soybean breeding programs, which contributes to the generation of new cultivars with different traits to adapt to the different environments. Besides, the same authors affirm that the genetic variability level of soybean cultivars has remained constant over the years in Brazil.

For the level of technology used, it can be observed that cultivars with the IPRO technology presented the shortest distance between the cultivars (dii' = 0.092) between minimum observed (Table 3). This same technology also presented the shortest distance (dii'

= 0.98) when observed maximum distances are indicating less genetic variability. The smallest found variability in cultivars with the IPRO technology (intact BtRR2) is related to the reduced number of cultivars as well as the time of insertion of this technology in the Brazilian market, which dates back to the 2013/2014 season.

Tocher method grouping analysis (Table 4), generated based on dissimilarity measures, ranked soybean cultivars into two groups, the group I, and the group (II) composed by cultivar 97R73 RR (8). This fact has already been observed and reported in the literature by Almeida et al. (2011). However, other authors observed greater group formation as presented by Santos et al. (2011), 4 distinct groups, and Shadakshari et al. (2011), 10 groups; however, this last one evaluated soybean genotypes available in the Indian market.

Table 4. Soybean grouping cultivars indicated by the Tocher method, from the dissimilarity matrix of Euclidean distance estimated by phenotypic characters between soybeans, UFLA, Lavras - MG, 2015.

Group	Soybean cultivars
I	CG 68 RR; M 7110 IPRO; RK 6813 RR; FPS SOLAR IPRO; FPS JUPITER RR; FPS ANTARES RR; FPS ATALANTA IPRO; NK 7059 RR; V-TOP; BMX POTENCIA RR; FPS PARANAPANEMA RR; V-MAX; FPS URANO RR; FPS SOLIMÕES RR; BMX FORÇA RR; NS 7200; NA 5909 RG; RK 5813 RR; CD 215 RR; CD 202 RR; LG 60163 IPRO; NS 5151 IPRO; 5D 615 RR; NS 5106 IPRO; 95R51 RR; 5D 6215 IPRO; AS 3575 IPRO; FPS NETUNO RR; BRSMG 760 SRR; BRSMG 68 VENCEDORA; BRSMG VALIOSA RR; BRSMG 752S; BRSMG 780 RR; TMG 7161 RR; 5D 690 RR; FMT0860.346/1; CD 250 RR; TMG 123 RR; CG 67 RR; BMX DESAFIO RR; CG 7464 RR; NS 7114; BRS 213; AS 3610 IPRO; 97R21 RR; CG 7665 RR; BRSMG 772; BRSMG 810C; TMG 7262 RR; SYN 13610 IPRO; BMX PONTA IPRO; CD 2737 RR; M 7211 RR; NS 7300 IPRO; CD 238 RR; P98Y11; CD 237 RR; NS 7100; CG 8166 RR; BRS FAVORITA RR; 5G 770 RR; LG 60177 IPRO; BRSMG 820 RR; TMG 1176 RR; Anta 82 RR; BRSMG 800 A; 5G 830 RR; NS 7209 IPRO; TMG 1181 RR; BRS MG 790 A; TMG 127 RR; TMG 1179 RR; BRSMG 850 GRR; FMT0871.422/3 and FMT0861.708/2
II	97R73RR

Grouping through UPGMA method also established the formation of two distinct groups (Figure 1), with the same constitution presented by Tocher's optimization method. Similarities among groupings were already described in the literature by some authors. Santos et al. (2011) concluded that UPGMA methods and Tocher method were also concordant with each other by grouping 48 soybean genotypes into four groups.

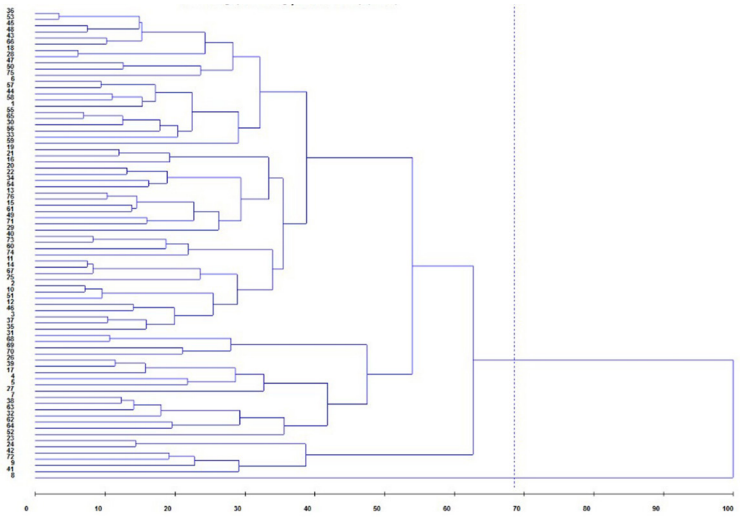

Figure 1. Genetic distance dendrogram of soybean cultivars obtained by UPGMA grouping analyses, by phenotype characters, UFLA, Lavras - MG, 2015.

The formation of groups, regardless of the method of grouping, is of relevance in the choice of parents since its future hybrid combinations to be established should be based on the magnitude of their dissimilarities and individual parent potential being considered as a starting point in breeding programs (Vieira et al., 2007; Cruz et al., 2014). Assembled cultivars in more distant groups are dissimilar and may be considered as a promising artificial cross (Peluzio et al., 2009). In this statement, the presence of the cultivar 97R73 RR (8) in isolated groups for both methods of grouping indicates that this parent can provide genetic gains in the selection after the hybridizations.

To not restrict the genetic variability and, therefore, avoid negative impact in gains to be obtained by selection, it is recommended by Cruz et al. (2014) a non-involvement of individuals of the same pattern of dissimilarity at crosses. The best hybrid combinations to be tested in the breeding program should involve parents involving high average performance and variability for the traits to be improved (Carpentieri-Pípolo et al., 2000).

Relative contribution of each character to the genetic dissimilarity, according to Singh (1981), showed that the characters with lower relative contribution were the lodging of plants (0.12); plant height (5.30); first legume insertion height (0.32); one-thousand seed mass (0.50), and maturity number days (2.31), representing only 8.55% of the relative contribution (Table 5).

Table 5. Estimates of the relative contribution of variables to the genetic diversity, using criteria of Singh (1981) based on Euclidean distance (dii'), to phenotypic characters, UFLA, Lavras - MG, 2015.

Variable	Relative contribution (%)
Grain yield	91.45
Plant lodging	0.12
Plant height	5.30
Height insertion of first legume	0.32
One-thousand seed mass	0.50
Full maturity	2.31

For Rigon et al. (2012), the low contribution of these characters especially when it refers to plant height, first legume insertion height, and plant lodging to distinguish between the genotypes are mainly due to the fact that genetic breeding of these characters in question was intensified in the soybean crop in recent years because they are directly related to grain yield.

The strongest characteristic, and consequently, the greatest contribution to genetic divergence was grain yield with contribution higher than 90.0%. Similar results were found by Oliveira et al. (2014) evaluating genetic divergence among soybean genotypes in which was also observed higher relative contribution of grain yield character. According to Peluzio et al. (2009), grain yield characterization is of fundamental importance in breeding, since in the selection of parents with higher average productivity in breeding they are more likely to obtain elite lineages.

In the study of genetic diversity based on enzymatic markers or biochemical markers, as it is known by most researchers (Hoffmann and Barroso, 2006), polymorphism was observed in all enzymatic systems selected enabling the use of isoenzymatic technique analysis in discrimination and the study of genetic variability among soybean cultivars.

Enzyme systems varied in locus number, which for the enzymes alcohol dehydrogenase, esterase, superoxide dismutase, and peroxidase 1, 3, 3, and 4 loci were observed, respectively, being checked higher polymorphism when peroxidase enzyme was used. Enzymes are

considered important markers for the characterization of cultivars because they are easily detected and frequently expressed in several parts of plant materials being used in researchers for studies of diversity of cultivars in different cultures.

For the enzyme alcohol dehydrogenase, polymorphism was verified between millet cultivars by Mendonça Neto et al. (2013) when using this enzyme as a marker, although the same fact was observed by Vieira et al. (2009), working with soybean and Menezes et al. (2008) working with breeding lines and hybrids of maize. Using esterases as markers, by Vieira et al. (2009), polymorphism was observed for soybean cultivar separation, as well as by Ferreira et al. (2009) in *Gladiolus* and Mendonça Neto et al. (2013) in millet crop. The same fact is reported by Vieira et al. (2009) using the enzymes superoxide dismutase and peroxidase in studies with soybean cultivar polymorphisms observed for cultivar separation.

Regarding the study of genetic diversity considering the cutting line, six groups of cultivars were formed (Figure 2). From the formed groups, three of them allowed discrimination of an individual form, thus constituting group 6: CG 8166 RR, group 5: FPS Jupiter RR, and group 4: BRSMG 780 RR, suggesting that these are the most different cultivars among the studied ones and potential parents when the interest is crossing between groups more different. The other groups were constituted by groups of cultivars according to their similarity constituting groups 3, 2, 1, with (12), (35) and (26) cultivars, respectively.

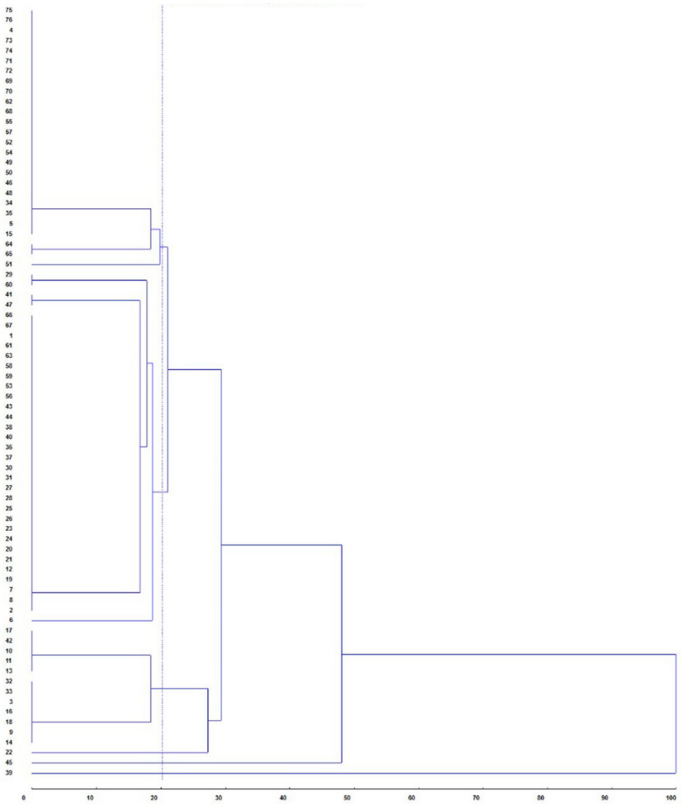

Figure 2. Genetic distance dendrogram of soybean cultivars obtained by UPGMA grouping analyses, by isoenzymatic markers, UFLA, Lavras - MG, 2015.

Even though analyses (phenotypic characters and enzymatic markers) share some results, on the other hand, they have revealed differences. The most different pairs of cultivars found through the Euclidean distance (phenotypic trait) differ from those obtained by the Jaccard coefficient (enzymatic markers). However, it can be verified that in both the cultivars, RR was the most different related to the others. Differences were also observed in the grouping of cultivars of the TMG and Nidera program in which the genotypes of these programs were more similar when analyzed by enzymatic markers being present in groups I and II.

One of the factors that make difficult the occurrence of association between phenotypic traits and enzymatic markers is that the variation detected by enzymatic markers is not adaptive, and therefore, not subject to selection unlike the phenotypic traits that are subject to both natural and artificial selection, in addition to suffering great environmental influence (Vieira et al., 2005).

However, the use of phenotypic traits and enzymatic markers provide a complete picture of the diversity present in the genotypes evaluated. The best way to identify differences between genotypes is the combined use of enzymatic markers and phenotypic traits promoting an improvement in the results (Singh et al., 1991). Accordingly, Chioratto et al. (2007) suggest that the phenotypic traits and enzymatic markers should be used together in diversity studies, contributing to the reliability of results and correct understanding of the relationship between the accesses.

In addition to the genetic divergence for the choice of the parents for the hybridization program and subsequent selection of higher individuals in segregating generations, the performance *per se* of parents, as well as the allelic complementarity allele between them, should be considered (Souza et al., 2005). With crossing between groups that present a greater distance what is expected is obtaining superior individuals with heterosis manifestation (Borém, 2006).

CONCLUSIONS

Use of phenotypic traits in the study of genetic diversity led to the formation of two groups, discriminating individually the cultivar 97R73 RR. Using enzymatic markers, the soybean cultivars were classified into six distinct groups, discriminating the cultivars CG 8166 RR, FPS Jupiter RR, and BRS MG 780 RR individually.

The characteristic with the greatest contribution to genetic divergence was grain yield with contribution higher than 90.0%.

Cultivars carrying the RR technology were the most different when compared to conventional and IPRO cultivars.

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