Phenotypic and molecular diversity among soybean cultivars as a function of growing season

Amilton Ferreira da Silva[1], Tuneo Sediyama[2], Éder Matsuo[3,*], Francisco Charles do Santos Silva[2], Cosme Damião Cruz[4], Aluízio Borém[2] and André Ricardo Gomes Bezerra[2]

[1]Departamento de Ciências Agrárias, Universidade Federal de São João del-Rei, Campus Sete Lagoas, Rodovia MG 424, Km 47, Caixa Postal 56, CEP 35701-970, Sete Lagoas, Minas Gerais, Brazil. [2]Departamento de Fitotecnia, Universidade Federal de Viçosa, Av. PH Rofs, s/n, Campus Universitário, CEP 36570-900, Viçosa, Minas Gerais, Brazil. [3]Instituto de Ciências Extas e Tecnológicas, Universidade Federal de Viçosa, Campus Rio Paranaíba, Rodovia MG 230, Km 7, Caixa Postal 22, CEP 38810-000, Rio Paranaíba, Minas Gerais, Brazil. [4]Departamento de Biologia Geral, Universidade Federal de Viçosa, Av. PH Rofs, s/n, Campus Universitário, CEP 36570-900, Viçosa, Minas Gerais, Brazil, *Corresponding author, E-mail: edermatsuo@ufv.br

ABSTRACT

The identification of divergence among cultivars adapted to specific soil and climatic conditions is of fundamental importance for the realization of promising hybridizations. This diversity can be accessed through phenotypic characteristics and molecular markers. In this sense, the objective of this study was to evaluate genetic diversity of soybean cultivars in the summer and winter from agromorphological traits and molecular markers. Two experiments were conducted, one in the summer (2011) and another in the winter (2012). The experiments were conducted in a randomized block design with four replications. Six widely grown cultivars were used and they showed striking differences in regards to morphological markers. The cultivars are: M 7211 RR, TMG 123 RR, TMG 1176 RR, M 7908 RR, TMG 127 RR and TMG 7188 RR. During the development of the plant, as well after harvesting were evaluated 18 traits. The DNA of six cultivars was amplified with 16 primers (microsatellite markers) flanking microsatellite regions in soybean located in sixteen of the twenty soybean linkage groups. Genetic dissimilarity between cultivars from agromorphological traits varies depending on the growing season. The molecular markers showed genetic variability between cultivars with different results for clusters formed from the agronomic characters. Thus, both phenotypic and the molecular data proved to be informative tools to characterize the existing conflict between soybean cultivars.

Key words: *Glycine max*, Genetic diversity, SSR marker, Cluster, multivariate analysis.

INTRODUCTION

The knowledge of genetic diversity and the relationship between improved cultivars are of great importance for the continuous improvement of crops. Genetic diversity ensures protective measures against future problems such as pests or diseases and provides a basis for genetic gains (Bertini et al., 2006). In the determination of the soybean genetic diversity has been verified the use of morphological, agronomic, biochemical, physiological and molecular characteristics.

The study of genetic diversity through agronomic traits is of great interest in genetic improvement in view of its economic importance and the need to succeed in the proper choice of superior hybrids. Liu et al. (2011) observed that only nine agronomic traits were sufficient to allocate 91 soybean lines into two distinct groups, which corresponded to their geographical origins. Almeida et al. (2011) evaluated eight agronomic traits to promote the combined yield as a function of genetic dissimilarity, and facilitate the choice of most divergent parents. Santos et al. (2011) estimated the genetic diversity among 48 soybean genotypes using 17 phenotypic traits and were able to identify promising crosses aiming to increase grain production. Rigon et al. (2012) evaluated 18 soybean cultivars through six quantitative traits and observed the identification of dissimilar genotypes for potential artificial breeding.

However, not always a remarkable morphological difference, such as leaf type, can mean high genetic similarity between cultivars. For plants with a relatively narrow base such as soybeans and taking into consideration the availability of hundreds of cultivars in the market, these characteristics may not be sufficient to separate different genotypes. Due to this limitation, DNA molecular markers have been widely used. Molecular markers are stable and proven to be informative and can be used in genetic characterization such as analysis of the degree of divergence and discrimination of accessions in a gene bank (Rongwen et al., 1995; Nelson and Li 1998).

Among the DNA markers, the microsatellite (SSR) markers have been of invaluable importance to estimate the genetic diversity in soybean due to its abundance, high degree of polymorphism, co-dominant traits, PCR based detection and the fact that they have known positions in the genome (Priolli et al., 2010). Diwan and Gregan (1997), using 20 SSR markers, were able to distinguish the 35 genotypes that contributed with about 95% of the alleles present in current US cultivars. Song et al. (1999), using only 13 SSR markers, were able to genetically differentiate morphologically similar cultivars, and Rongwen et al. (1995) concluded that 10 to 15 SSR locos are suitable to distinguish between closely related cultivars.

In recent years, microsatellites have been considered ideal for the characterization and evaluation of genetic variability and are the most widely used tools in plant breeding. By using these markers, it is possible to organize the active germplasm of a breeding program in gene pools, facilitating the selection and thus reducing the number of combinations to be made by breeders.

For the interpretation of results of both molecular and phenotypic data, clustering methods can be used to identify and divide groups of samples into subgroups according to their similarities or differences. The technique is carried out after obtaining a dissimilarity matrix, and based on this matrix, the groups are identified and the homogeneity and the heterogeneity among them are obtained (Cruz et al., 2011).

The use of phenotypic traits and molecular markers may provide a more complete view of the genetic diversity of materials. Singh et al. (1991) reported that the best way to identify the divergence between genotypes is the combined use of molecular markers and agromorphological characters, providing a complement to the results. Thus, the aim of this study was to evaluate the genetic diversity of soybean in the summer and winter using agromorphological traits and molecular markers.

MATERIALS AND METHODS

The study was conducted in the Soybean Breeding Program greenhouse located at the Department of Plant Science from the Universidade Federal de Viçosa. Two experiments were conducted, one in the summer (2011) and another in the winter (2012). The experiments were conducted in a randomized block design with four replications. Each experimental unit consisted in a mean of two plants cultivated at a 2.5 L pot with soil.

Six widely grown cultivars (M 7211 RR, TMG 123 RR, TMG 1176 RR, M 7908 RR, TMG 127 RR and TMG 7188 RR) were used and they showed striking morphological markers differences. All cultivars are recommended for the central region of Brazil.

During the development of the plants and after harvesting, the following assessments were made by adopting the stage of soybean development proposed by Fehr and Caviness (1977):

Duration of the vegetative stage (DEV): number of days was d to the R1 stage in which the first flower bud emerges on any node of the main stem.

Plant height at flowering (APF): surface measured from the soil until the last node of the main stem.

Number of nodes on the main stem at flowering (NNF): all nodes on the main stem were counted at the R1 stage.

Number of days to maturity (NDM): the number of days between emergence and the R8 stage was counted (95% of mature pods).

Plant height at maturity (cm) (APM): surface measured from the soil until the last node on the main stem in R8.

First pod height (cm) (AIV): surface measured from the soil until the insertion of the first pod of the main stem.

Number of nodes on the main stem at maturity (NNM): all nodes on the main stem were counted at the R8 stage.

Number of pods with 1 grain (V1G): After harvesting, all pods with one grain in each plant were counted.

Number of pods with 2 grains (V2G): After harvesting, all pods with 2 grains in each plant were counted.

Number of pods with 3 grains (V3G): After harvesting, all pods with three grains in each plant were counted.

Number of pods with 4 grains (V4G): After harvesting, all pods with 4 grains in each plant were counted.

Number of pods per plant: (NVP): After harvesting all pods from each plant were counted.

Number of grains per plant (NGP): After thrashing the pods, all grains from each plant were counted.

Number of grains per pod (NGV): It was obtained by dividing number of grains by number of pods per plant.

Weight of 100 grains (M100): It was acquired by the average weight of 100 grains from each cultivar.

Grain yield per plant (PGP): After threshing, every seed from each plant was weighed.

Harvest index (IC): Obtained by dividing the weight of the seeds by the total mass of the whole plant.

Length/width ratio of the leaf (RCL): the length and width of a leaf located in the node 4 counted from top to down in the R5 stage was measured to obtain the relationship.

All measurements were performed in two plants in order to later retrieve plot average.

Molecular analysis

The molecular analyzes were performed at Bioagro (Institute of Biotechnology Applied to Agricultural) located in the Universidade Federal de Viçosa. A sa mple of each cultivar was grounded and subjected to DNA extraction, according to the protocol described by Schuster et al. (2004). The DNA of six cultivars was amplified with 16 primers (SSS Marker) which flank microsatellites regions in soybean developed by Cregan et al. (1999). The primers (Satt022, Satt168, Satt191, Satt251, Satt263, Satt274, Satt277,Satt313, Satt335, Satt389, Satt463, Satt468, Satt571, Satt578, Satt581 and Satt632) were selected according to their distribution in the soybean genetic map (Song et al., 2004), located in sixteen of the twenty soybean linking groups. Information on the marker sequence for all microsatellite markers can be found at the Soybase website (http://www.soybase.org).

The DNA extraction was performed using the Wizard Genomic DNA Purification Kit (Promega). The amplification reactions were performed in volumes of 15 μL containing 30 ng of DNA, 10mM of Tris HCl/KCl (pH 8.3), 2.0 mM of $MgCl_2$, 100 μM deoxynucleotides (dATP, dCTP, dGTP e dTTP), 067 μM of each primer and 1U of Taq DNA polimerase (Phoneutria). The PCR reaction was programmed for an initial step at 94 ºC for 2 minutes, 37 cycles consisted of three steps (94 ºC for 30 seconds, 54 ºC for 30 seconds and 72 ºC for 45 seconds), followed by a final step at 72 ˚C for 7 minutes.

The amplification products were subjected to vertical electrophoresis at 10% polyacrylamide gel in a 1X TAE running buffer at 130 V, for 4 hours. In order to view the fragments, the silver staining protocol was performed. Initially, the gel was subjected to the fixing solution (10% of ethanol and 0.5% of glacial acetic acid), stirred for 10 min. Then, the solution was discarded, and the 0.2% silver nitrate solution was added under stirring for 10 min. Then, the silver nitrate solution was discarded, the gel was washed with purified water and the developing solution (0.75 M NaOH and 0.6% formaldehyde) was added under stirring. When the fragments become visible, a 60% ethanol solution was added to stop the reaction. Finally, the gels were photographed for analysis.

Statistical analysis

For quantitative traits, the genetic dissimilarity between all pairs of cultivars was initially estimated by the Mahalanobis Widespread Distance ($D^2_{ii'}$). After obtaining the dissimilarity matrix between cultivars, a cultivar grouping was performed by the Hierarchical Method of the Average Linking Between Group (UPGMA), the Tocher Optimization Method and the two-dimensional scatter plot based on the canonical variables which were used in the study of genetic dissimilarity with the intent of identifying similar cultivars. The relative contribution of features for diversity was based on the method of Singh (1981).

The genetic relationships among cultivars obtained by SSR markers were evaluated by means of dissimilarity matrix, constructed with use of the complement of the similarity index for codominant and multi-allelic data. Based on dissimilarity estimates, the cultivars were grouped by the UPGMA hierarchical method and the Tocher Optimization Method, and were presented in a 2D scatter chart. Finally, the correlation between matrixes is calculated by the t and Mantel test. All analyzes were performed with the Genes Program (Cruz 2013).

RESULTS AND DISCUSSION

Phenotypic diversity - summer

The shortest distance was observed between the TMG 123 RR and TMG 1176 RR (154.34) cultivars (Table 1A), which shared a common characteristic (lance type leaves). While the most distant cultivars were TMG 127 RR and TMG 7188 RR (54119.99), with the latter showing a small distance from cultivar M 7908 RR (3475.60).

By the UPGMA clustering, the formation of three groups was observed, resulting in three pairs of similar cultivars (TMG 123 RR and TMG 1176 RR; M 7211 RR and TMG 127 RR; M 7908 RR and TMG 7188 RR) (Figure 1A). If the phenotypic characteristics of the cultivars are observed, a more striking characteristic can be considered for each of the following group: lance-shaped leaves (characteristic linked to the occurrence of pods with four grains), lower relative maturity group and largest relative maturity group, respectively. The relative maturity group difference for cultivars planted in the same place, at the same time, determines the vegetative and reproductive period as well as the influence on the production components. Thus, for example, cultivars from a larger group of relative maturity are able to produce more nodes and more seed per pods and, therefore, greater yield.

The same grouping by the UPGMA method was observed by the Tocher Optimization Method (Table 2A). This grouping is consistent with the observed morphological characters. Thus, although a limited group of cultivars, different potential parents could be observed in the case of hybridization. The scatter chart (Figure 2A) was obtained by calculating the canonical variables and the same three groups were formed.

According to the relative contribution analysis by the method of Singh (1981), among traits (Table 3), the most influential parameter, and thus the most intense contribution for the genetic divergence was number of pods per plant (NVP) (47.00 %), followed by number of pods with 4 grains (V4G) (21.02 %) and number of 3 grains (N3G) (15.69 %). Summer is the recommended period for soybean seeding thus pod production is relatively large under ideal management conditions. Peluzio et al. (2009), working only with the total number of pods along with six other characteristics, observed that the number of pods per plant was the largest contributor to genetic similarity (80.17%) among the 14 cultivars.

Phenotypic diversity - winter

The shortest distance was observed between cultivars TMG 1176 RR and M 7908 RR (2085.25), while the longest distance was observed between TMG 123 RR and TMG 7188 RR (241465.20) (Table 1B). By the matrix, it is possible to observe the different behavior of the cultivars in relation to cultivation in the summer.

By using the UPGMA grouping technique (Figure 2B), it was possible to observe the formation of two groups,

only the TMG 7188 RR cultivar was separated from the others. In the winter, not all the conditions are favorable for the development of soybean plants, thus some cultivars which would not stand out in the summer may be superior in relation to the others in the winter, by interacting with this cultivar environment. In this case, cultivar TMG 7188 RR has the highest relative maturity group (8.8) from this set of cultivars, which becomes an advantage, since in the winter, the photoperiod is the lowest, i.e. the nights are longer, which induces early flowering. So cultivars considered to be precocious in the normal planting season become even more precocious in the winter, modifying many features like plant height and number of nodes, which affects the production of components and productivity.

The same two groups were formed by the Tocher Optimization Method (Table 2B) confirming cultivar behavior change regarding planting time. Thus, by comparing the groups in both seasons, the summer groups showed more consistency with the observed characteristics, since it is the period in which plants can express their potential. But it is also important to make the out of season analysis to verify crop adaptability to harsh conditions

As for the summer variables, the 2D scatter chart obtained by calculating the canonical variables (Figure 3B) showed the same groups obtained by UPGMA and Tocher. Thus, using three different clustering methods, it is possible to obtain a reliable result regarding the dissimilarity of these cultivars considering the planting season.

Table 1. Dissimilarity matrix among six soybean cultivars.

A: Based on the Mahalanobis distance from 18 agronomic traits evaluated in the summer

	M 7211RR	TMG 123RR	TMG 1176RR	M 7908RR	TMG 127RR	TMG 7188RR
M 7211 RR	0					
TMG 123 RR	13468.10	0				
TMG 1176 RR	12121.54	154.34	0			
M 7908 RR	12647.91	15729.61	15399.48	0		
TMG 127 RR	4119.63	23691.83	21683.38	31029.86	0	
TMG 7188 RR	28571.32	27177.08	27386.02	3475.60	54119.99	0

B: Based on the Mahalanobis distance from 18 agronomic traits evaluated in the winter

	M 7211RR	TMG 123RR	TMG 1176RR	M 7908RR	TMG 127RR	TMG 7188 RR
M 7211 RR	0					
TMG 123 RR	30880.93	0				
TMG 1176 RR	5043.95	15320.86	0			
M 7908 RR	5659.07	12191.15	2085.25	0		
TMG 127 RR	21438.83	4160.71	9625.26	8785.68	0	
TMG 7188 RR	102485.80	241465.20	136553.70	149445.00	206999.00	0

C: calculated from the arithmetic complement of the simple coincidence coefficient

	M 7211RR	M 7908RR	TMG 123RR	TMG 127RR	TMG 1176RR	TMG 7188RR
M 7211 RR	0					
TMG 123 RR	0.21053	0				
TMG 1176 RR	0.63158	0.63158	0			
M 7908 RR	0.68421	0.47368	0.57895	0		
TMG 127 RR	0.73684	0.84211	0.57895	0.63158	0	
TMG 7188 RR	0.84211	0.63158	0.63158	0.50000	0.63158	0

In the winter experiment, the relative contribution analysis (Singh 1981) (Table 3) showed that the variables that contributed the most to the genetic divergence were the number of pods with 2 grains (V2G), followed by the number of grains per plant (NGP), and the number of pods per plant (NVP) and number of pods with 2 grains (V2G). From these data, it is possible to observe that the contribution of the production components may vary depending on the season. In the summer, the greatest contribution came from the total number of pods (NVP), but, in the winter, the number of pods with 2 grains (V2G) offered greater contribution (30.8%) while, in the summer, it was extremely low (1.69%). Another component with high contribution in the winter season was the number of grains per plant (NGP) with 22.2%, whereas, in the summer, its contribution was only 15.1%.

Molecular diversity

The matrixes of genetic dissimilarity estimated between cultivars (Table 1C), based on the markers used, ranged from 0.210 to 0.842, the shortest distance was observed between M 7211 RR and TMG 123 RR (0.21053) and the longest distance between M 7211 RR and TMG 7188 RR (0.84211) and among cultivars TMG 123 RR e TMG 127 RR, which had the same estimated distance. This result indicates considerable genetic variability among cultivars. Priolli et al. (2004) studied the diversity of eight cultivars from Brazilian breeding programs and found that the diversity index varied from 0.435 to 0.809 with an average value of 0.632. The authors concluded that the Brazilian soybean germplasm has maintained constant genetic variability, expansion and crop improvement in the last 30 years. Vieira et al. (2009) observed genetic dissimilarity between 53 cultivars, ranging from 0.02 to 0.73, with an average of 0.47. By estimating the genetic diversity in RR soybean cultivars, Villela (2014) observed genetic distances between 0:46 and 9.79, obtained by means of agronomic traits and estimated from the Euclidean distance, indicating the presence of genetic variability between the evaluated RR soybean cultivars from different soybean breeding programs in Brazil.

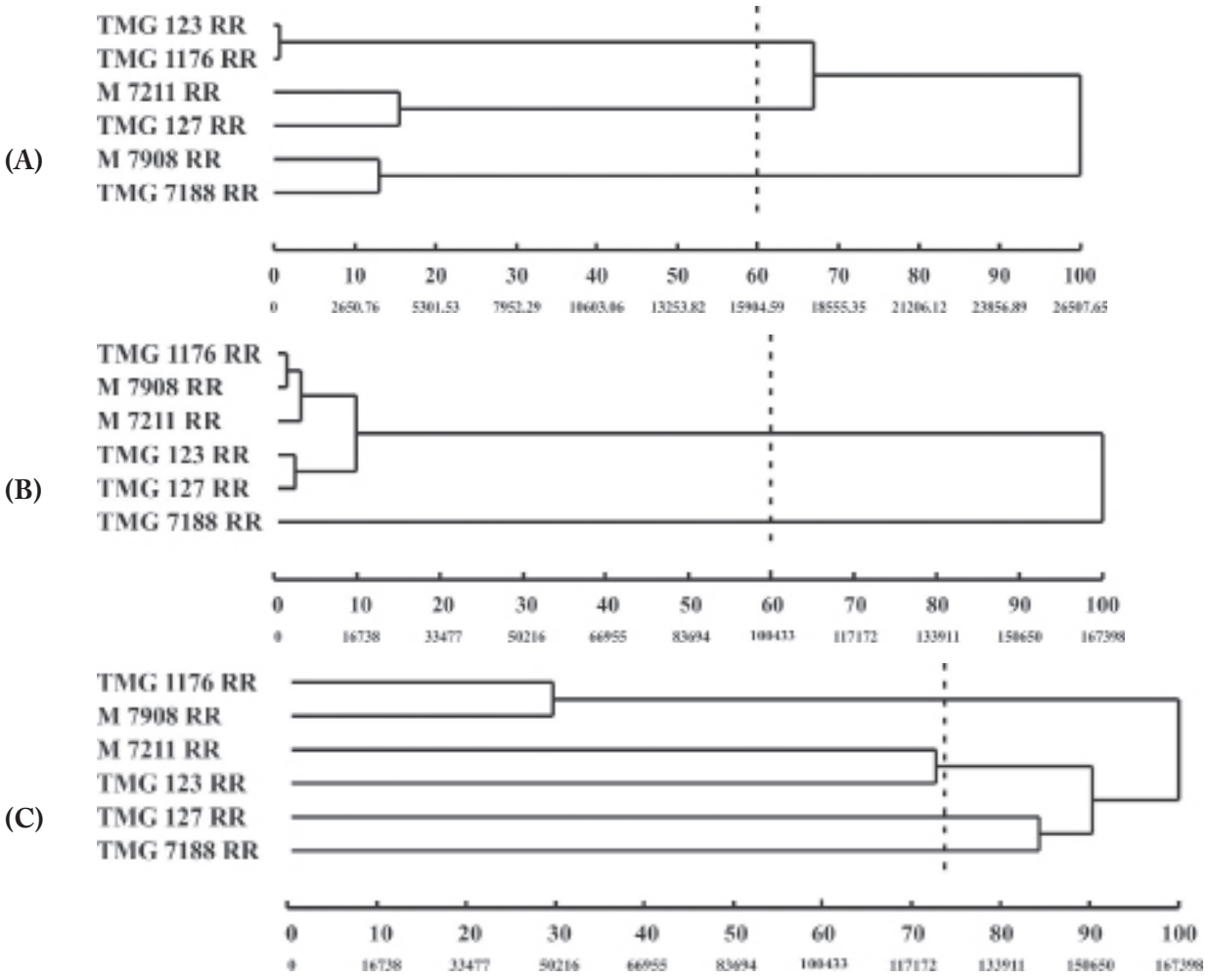

Figure 1. Dendrograms obtained by the UPGMA method of six varieties. (A) Based on the Mahalanobis generalized distance as a measure of dissimilarity from 18 agronomic traits measured in the summer; (B) Based on the Mahalanobis generalized distance as a measure of dissimilarity from 18 agronomic traits measured in winter; and (C) Based on microsatellite markers. Cophenetic correlation = 0.70; 0.92 and 0.81, respectively.

The cluster analysis by the UPGMA method is represented by the dendrogram (Figure 1C). Cultivars M 7211 RR and TMG 123 RR formed a group, M 7908 RR and TMG 7188 RR formed another group, and finally, TMG 127 RR and TMG 1176 RR each one formed an isolate group. Grouping by UPGMA is currently the most widely used method in genetic diversity studies (Dias 1998; Cross and Aries 2006), this algorithm does not consider the group subdivision structure, giving equal weight to each individual group, and calculates the average similarity of an individual who intends to join the existing group (Meyer 2002).

The fact that cultivars of the same enterprise do not form a group demonstrates that there is variability within breeding programs, since companies currently work aim at different regions of adaption. According to Priolli et al. (2004) soybean breeding programs in Brazil have greater intra rather than intergroup variability. Bonato et al. (2006) also presented molecular analysis results showing that the soybean germplasm used by Brazilian breeding programs has maintained a generally constant level of genetic diversity in recent years and the relative heterogeneity within some of these programs.

The Tocher Optimization Method resulted in the formation of three groups (Table 2C), the first with three cultivars, two in another group and a third with a single cultivar. This clusters was different of groups formed from UPGMA method. The Tocher Optimization Method identifies the pair of more similar individuals to form the initial group and from there it is possible to evaluate the possibility of adding new individuals to the criteria which establishes that the average intra-group distance must be less than the average intergroup distance (Cruz and Carneiro 2006).

Through the scatter graph, it is possible to better observe the distance between cultivars (Figure 2C). The greatest difference here compared to the UPGMA and Tocher clustering methods was the proximity between cultivars M 7908 RR and TMG 1176 RR, which were not grouped into any of these methods. Cultivar M 7211 RR and TMG 123 RR continued to be relatively close, while TMG 127 RR and TMG 7188 RR were positioned alone.

In hierarchical clustering, the delimitation of the groups is made subjectively by observing the points of high level of change in the dendrogram, so different grouping patterns may occur. When there is no information about the genetic relationship between most genotypes, it is difficult to determine which clustering method is more accurate. Thus, when comparing the results from different criteria, erroneous inferences can be avoided (Arriel 2004).

For these six cultivars, only 8 of the 16 markers could be used to differentiate them. The markers were: Satt022, Satt274, Satt263, Satt335, Satt632, Satt463, Satt191 and Satt581. According to these markers' results, the minimum difference was between the M 7211 RR and TMG 123 RR cultivars, being differentiated only by Satt632. This marker is specifically linked to resistance to soybean cyst nematode (Soybase 2015), which the M 7211 RR cultivar does not have, while TMG 123 RR has resistance to races 1 and 3. On the other hand, the M 7211 RR and TMG 7188 RR cultivars showed difference by all eight markers. Results from these markers are in agreement with the dissimilarity matrix, where M 7211 RR and TMG 123 RR cultivars had a distance of 0.210, while M 7211 RR and TMG 7188 RR had a distance of 0.842. Thus, these markers can be considered highly informative for this group of cultivars. In studies performed by Passianotto (2010), it was possible to identify in the group of 23 microsatellite markers, six markers capable of discriminating a group of 48 soybean cultivars.

Despite having similar phenotypic characteristics, the two blade leaf cultivars did not form a group in any of the clustering method, even when some of the markers associated with QTL's for length, width and type of leaflet were used (Satt313, Satt022, Satt168 and Satt571). However, these markers are also associated with several other QTL's of agronomic importance. This demonstrates the importance of molecular analysis as a tool to support improvement, since phenotypically similar plants may often be potential parents.

Correlation between dissimilarity measures

The Mantel test showed low correlation between quantitative and molecular data, which again reinforces the need to use both of these characters in a diversity analysis. The correlation between the summer and the winter matrix (Figure 3A) was 0.57 (not significant, $P > 0.05$). By dividing the quadrants, it is possible to observe the genetic distance between cultivars that resisted the most in the region with the shortest distance (low diversity), while cultivar 6 showed the greatest distance from all the others, which determined higher genetic divergence combinations.

The correlation between dissimilarity measurements obtained from phenotypic characters data of summer and the matrix of the molecular data (Figure 3B) was relatively low, with a correlation coefficient of 0.42. It is possible to observe in this correlation that most of cultivar combinations were mainly explained by molecular data. Likewise, the correlation between winter and molecular data (Figure 3C) had the same behavior, and combinations of M 7211 RR x TMG 123 RR and TMG 123 RR x M 7908 RR showed less diversity at both times by the agromorphological and molecular data.

Indeed, there was a higher matrix correlation of molecular data with the matrix derived from the summer variables. Using the Mantel test, it was possible to see clearly that there is a correlation of 0.42 and 0.02 with summer and winter variables, respectively. This reinforces the increased reliability of the data obtained during the summer season,

Table 2. Grouping of six soybean cultivars by the Tocher Optimization Method

A: Using the Mahalanobis generalized distance from 18 agronomic traits evaluated in the summer	
Group	Cultivars
I	TMG 123 RR; TMG 1176 RR
II	M 7908 RR; TMG 7188 RR
III	M 7211 RR; TMG 127 RR
B: Using the Mahalanobis generalized distance from 18 agronomic traits evaluated in the winter	
Group	Cultivars
I	TMG 1176 RR; M 7908 RR; M 7211 RR; TMG 127 RR; TMG 123 RR
II	TMG 7188 RR
C: Using the dissimilarity through the arithmetic complement of the Simple Matching Coefficient	
Group	Cultivars
I	M 7211 RR; TMG 123 RR; M 7908 RR
II	TMG 127 RR; TMG 1176
III	TMG 7188

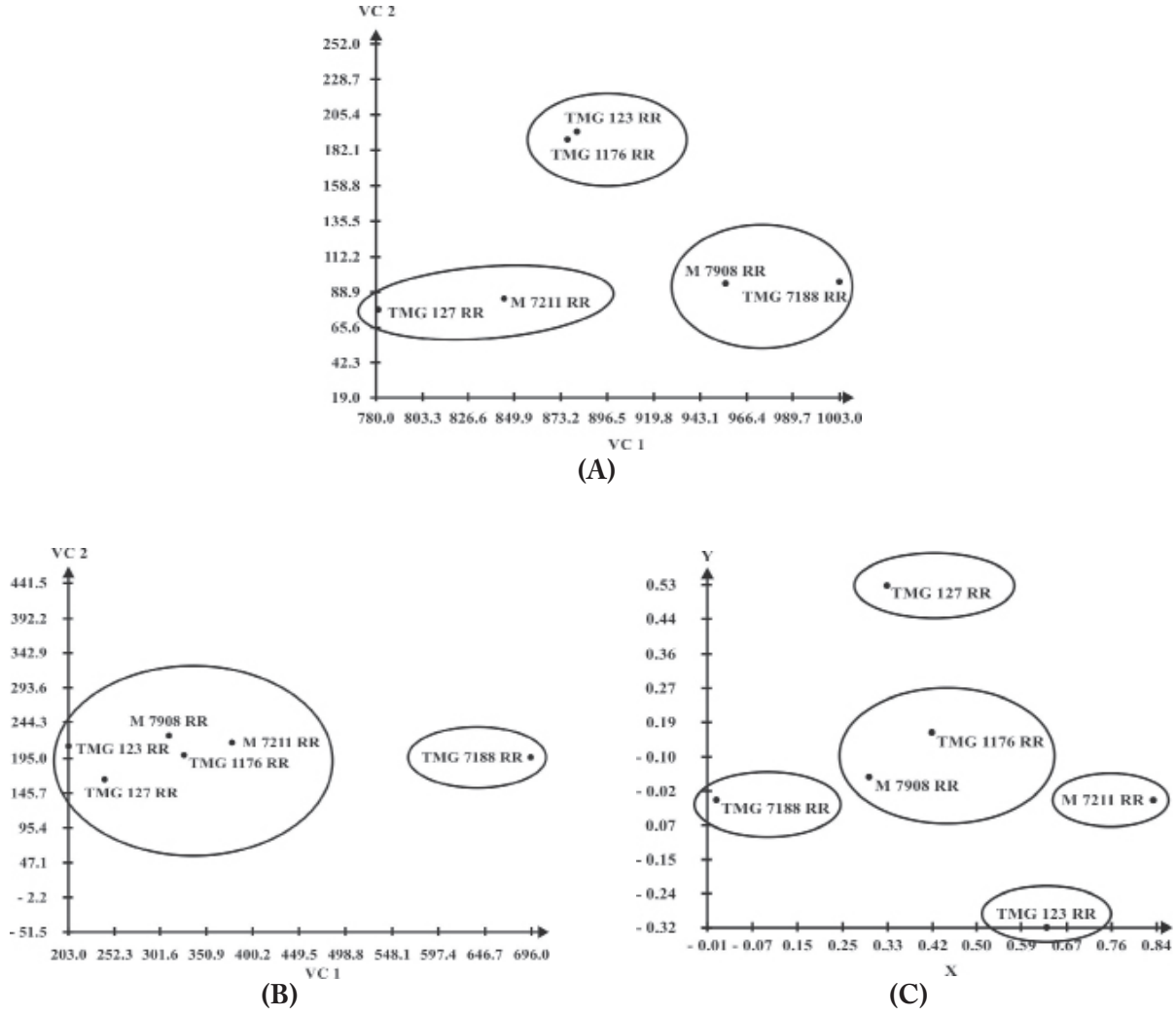

Figure 2. Graphic dispersion (2D scatter chart) of dissimilarity measures between six soybean cultivars. (A) Cultivated in the summer and based on canonical variables; (B) Cultivated in the winter and based on canonical variables; (C) Based on microsatellite DNA markers.

Table 3. Relative contribution, in percentage, of the variables on the genetic diversity of six soybean cultivars in the summer and winter using the method of Singh (1981).

Variable[1]	Summer (in %)	Winter (in %)
DEV	1.20	0.37
APF	0.07	0.54
NNF	0.61	0.70
NDM	0.39	0.12
APM	0.33	0.33
AIV	0.76	0.02
NNM	0.03	0.64
V1G	9.59	1.55
V2G	1.69	30.80
V3G	15.69	15.98
V4G	21.02	4.09
NVP	47.00	21.70
NGP	1.15	22.24
NGV	0.18	0.15
PGP	0.08	0.59
M100	0.05	0.02
IC	0.07	0.01
RCL	0.08	0.14

[1]DEV – Duration of the vegetative stage; APF – Plant height at flowering; NNF – Number of nodes in flowering; NDM – Number of days to maturity; APM – Plant height at maturity; AIV – Insertion height of 1st pod; MNM – Number of nodes on the main stem at maturity; V1G – Pods with 1 grain; V2G – Pods with 2 grains; V3G – Pods with 3 grains; V4G – Pods with 4 grains; NVP – Number of pods per plant; NGV – Number of grains per pod; PGP – Grain yield per plant; M100 – Weight of 100 grains; IC – Harvest index and RCL – Length/width ratio of the leaf.

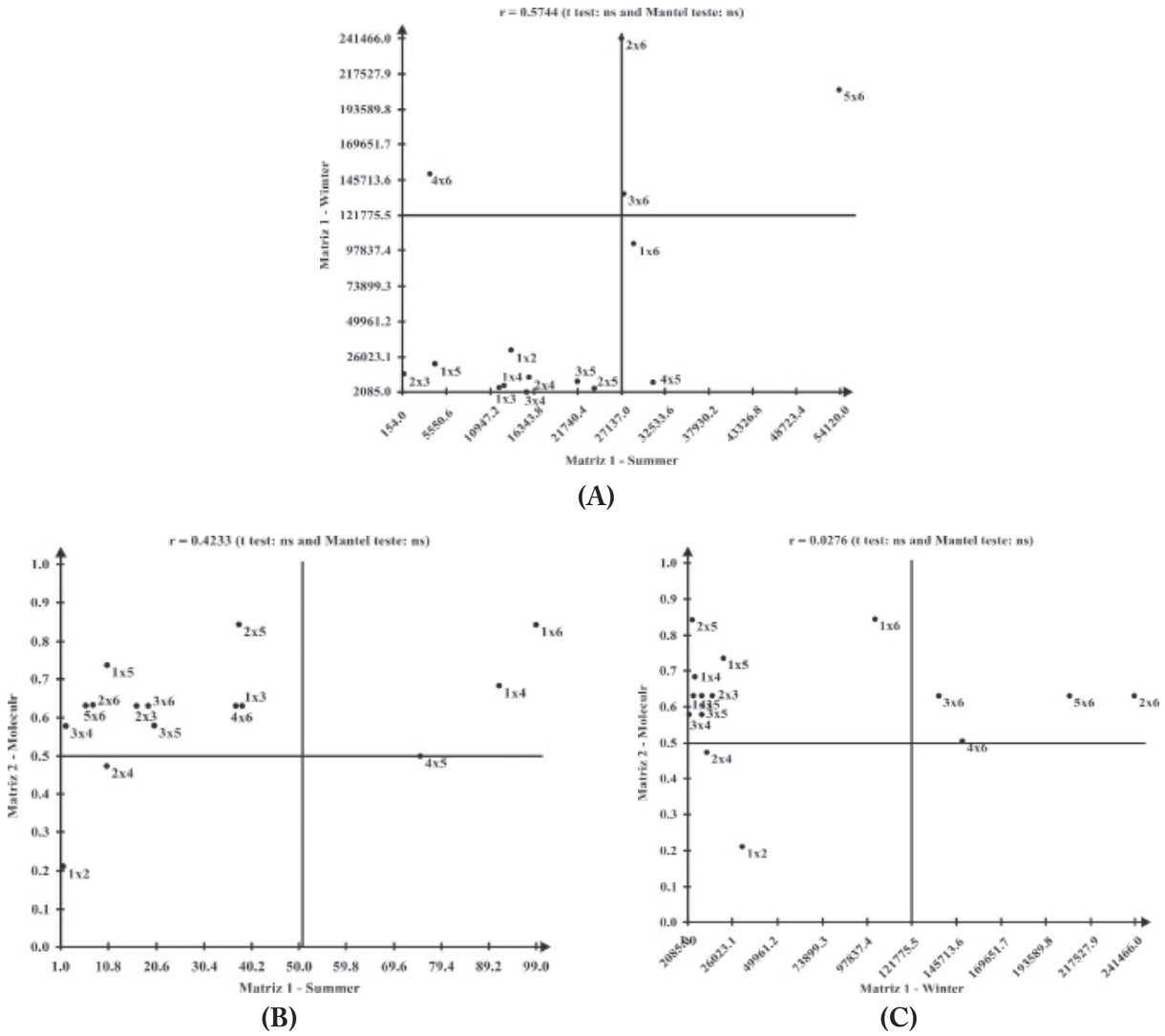

Figure 3. Correlations between matrixes with dissimilarity measures. (A) Obtained in the summer and winter evaluations between soybean cultivars; (B) Obtained in the summer evaluations and molecular data; and (C) Obtained in the winter evaluations and molecular data. Cultivars: 1 = M 7211 RR; 2 = M 7908 RR; 3 = TMG 123 RR; 4 = TMG 127 RR; 5 = TMG 1176 RR; and 6 = TMG 7188 RR.

which is suitable for soybean to express its potential. Therefore, a good analysis of diversity in soybeans must be made using both microsatellite genomic markers as agromorphological, in order to gain access to the diversity of the species in a more appropriate way, trying to access full genetic diversity.

Genetic variability between cultivars from agromorphological characters depends on the growing season, and means of molecular markers showed that the genetic variability among studied cultivars and formed clusters were different from those obtained with agronomic traits. Thus, both phenotypic and molecular data proved to be informative tools to characterize existing variability between soybean cultivars.

ACKNOWLEDGEMENT

We would like to thank FAPEMIG for the scholarship support.

REFERENCES

Almeida RD, Peluzio JM and Afférri FS (2011) Divergência genética entre cultivares de soja, sob condições de várzea irrigada, no sul do Estado Tocantins. Revista Ciência Agronômica 42: 108-115.

Arriel NHC (2004) Diversidade genética em gergelim (*Sesamum indicum* L.) a partir de marcadores moleculares (RAPD) e caracteres morfológicos e agronômicos. Thesis, Universidade Estadual Paulista.

Bertini CHCM, Schuster I, Sediyama T, Barros EG and Moreira MA (2006) Characterization and genetic diversity analysis of cotton cultivars using microsatellites. Genetics and Molecular Biology 29: 321-329.

Bonato ALV, Calvo ES, Geraldi IO and Arias CAA (2006) Genetic similarity among soybean (*Glycine max* (L.) Merrill) cultivars released in Brazil using AFLP markers. Genetics and Molecular Biology 29: 692-704.

Cregan PB, Jarvik T, Bush AL, Shoemaker RC, Lark KG, Kahler AL, Kaya N, Vantoai TT, Lohnes DG, Chung L and Specht JE (1999) An integrated genetic linkage map of the soybean genome. Crop Science 39: 1464-1590.

Cruz CD (2013) GENES - A software package for analysis in experimental statistics and quantitative genetics. Acta Scientiarum. Agronomy 35: 271-276.

Cruz CD and Carneiro PCS (2006) Modelos biométricos aplicados ao melhoramento genético. Editora UFV, Viçosa, v.2.

Cruz CD, Ferreira FM and Pessoni LA (2011) Biometria aplicada ao estudo da diversidade genética. Editora Suprema, Visconde do Rio Branco.

Dias LAS (1998) Análises multidimensionais. In: Alfenas AC (ed.) Eletroforese de isoenzimas e proteínas afins: fundamentos e aplicações em plantas e microorganismos. Editora UFV, Viçosa, p. 405-475.

Diwan N and Cregan PB (1997) Automated sizing fluorescent-labeled simple repeat marker to assay genetic variation in soybean. Theoretical and applied genetics 95: 723-731.

Fehr WR and Caviness CE (1977) Stage of soybean development. Iowa State University of Science and Technolog, Ames, 11p. (Special report 80).

Liu M, Zhang M, Jiang W, Sun G, Zhao H and Hu S (2011) Genetic diversity of Shaanxi soybean landraces based on agronomic traits and SSR markers. African Journal of Biotechnology 10: 4823-4837.

Meyer AS (2002) Comparação de coeficientes de similaridade usados em análises de agrupamentos com dados de marcadores moleculares dominantes. Dissertation, Universidade de São Paulo – Escola Superior de Agricultura Luiz de Queiroz.

Nelson RL and Li Z (1998) RAPD marker diversity among soybean and wild soybean accessions from four Chinese povinces. Agronomy Abstracts, ASA, Madison, p. 164.

Passianotto ALL (2010) Identificação molecular de cultivares de soja (*Glycine max* L. Merril) utilizando um sistema semi-automatizado de genotipagem. Dissertation, Universidade Estadual de Maringá.

Peluzio JM, Vaz-de-Melo A, Afférri FS, Silva RR, Barros HB, Nascimento IR and Fidelis RR (2009) Variabilidade genética entre cultivares de soja, sob diferentes condições edafoclimáticas. Pesquisa Aplicada & Agrotecnologia 2: 21-29.

Priolli RHG, Mendes-Junior CT, Sousa SMB, Sousa NEA and Contel EPB (2004). Diversidade genética da soja entre períodos e entre programas de melhoramento no Brasil. Pesquisa agropecuária brasileira 39: 967-975.

Priolli RHG, Pinheiro JB, Zucchi MI, Bajay MM and Vello, NA (2010). Genetic diversity among Brazilian soybean cultivars based on SSR loci and pedigree data. Brazilian Archives of Biology and Technology 53: 519-531.

Rigon JPG, Capuani S, Brito Neto JF, Rosa GM, Wastowski AD and Rigon CAG (2012) Dissimilaridade genética e análise de trilha de cultivares de soja avaliada por meio de descritores quantitativos. Revista Ceres 59: 233-240.

Rongwen J, Akkaya MS, Lavi U and Cregan PB (1995) The use of microsatellite DNA for soybean genotype identification. Theoretical and Applied Genetics 90: 43-48.

Santos ER, Barros HB, Ferraz EC, Cella AJS, Capone A, Santos AF and Fidelis RR (2011) Divergência entre genótipos de soja, cultivados em várzea irrigada. Revista Ceres 58: 755-764.

Schuster I, Queiroz VT, Teixeira AI, Barros, EG and Moreira MA (2004) Determinação da pureza varietal de sementes de soja com auxílio de marcadores moleculares microssatélites. Pesquisa Agropecuária Brasileira 39: 247-253.

Singh D (1981) The relative importance of characters affecting genetic divergence. The Indian Journal of Genetics e Plant Breeding 41: 237-245.

Singh SP, Gutiérrez JA, Molina A, Urrea C and Gepts P (1991) Genetic diversity in cultivated common beans: II. Markers based analysis of morphological and agronomic traits. Madison. Crop Science 31: 23-29.

Song QJ, Marek, LF, Schoemaker RC, Lark KG, Concibido VC, Delannay X, Specht JE and Cregan PB (2004) A new integrated genetic linkage map of the soybean. Theoretical and Applied Genetic 109: 122-128.

Song QJ, Quicley CV, Nelson RL, Carter TE, Boerma HR, Stracha JR and Cregan PB (1999) A selected set of trinucleotide simple sequence repeat markers for soybean cultivar identification. Plant Varieties and Seeds 12: 207-20.

Soybase (2015) SoyBase and the Soybean Breeder's Toolbox. http://www.soybase.org/. Accessed 19 March 2015.

Vieira ESN, Schuster I, Silva RB and Oliveira MAR (2009) Variabilidade genética em cultivares de soja determinada com marcadores microssatélites em gel de agarose. Pesquisa Agropecuária Brasileira 44: 1460-1466.

Villela OT (2014) Diversidade fenotípica e molecular de cultivares brasileiras de soja portadoras de gene RR. Dissertation, Universidade Estadual Paulista.

Received: May 28, 2015.

Accepted: July 23, 2015.

Published: November 19, 2015.