Genetic Diversity among Elite Brazilian Soybean Cultivars with Narrow Genetic Base

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ABSTRACT

This study was carried out to assess the genetic diversity among elite soybean cultivars with narrow genetic base using prediction techniques involving data from genealogy and agronomic traits. These predictions were then compared to the agronomic diversity observed by growing the derived cross progenies. The cultivars FT-Cristalina, Doko RC, IAC-12, UFV-10 were selected based on their genetic diversity obtained from molecular markers. The six possible hybrid progenies (F1) and the four cultivars were assessed in a glass house under controlled humidity and temperature, in a completely randomized design with four replications. The genetic diversity was predicted by genealogical inference and by analysis of the groups obtained using the Mahalanobis' generalized distance methodology based on the agronomic traits and Tocher's optimization method. The prediction techniques and the analysis of variance of the progenies indicated the presence of genetic diversity among the cultivars with narrow genetic base. The cultivar groups formed by genealogical inference were different from those obtained by Tocher' s optimization method. Genetic divergence evaluated by the application of prediction techniques using agronomic traits of the parents was similar to the agronomic divergence of the progenies. The prediction techniques were similar in screening only cultivars with maximum genetic divergence. It's possible to select soybean cultivars with genetic divergence among the elite materials. The crosses among soybean cultivars that maximize the genetic divergence varied according to the prediction technique used.

KEY WORDS: Genetic diversity, Selection.

INTRODUCTION

Selecting the best parents is essential to attain the objectives in a plant-breeding program. The selection may be based on the behavior of the cultivars themselves or on their genetic diversity, assessed from the parentage coefficient, or from agronomic or molecular traits. Cultivar selection to obtain segregating populations with wide genetic diversity for the traits of interest can be based on heterotic groups (Messmer et al., 1993). The identification of cultivars with greater genetic diversity allows the construction of linkage maps with greater band saturation, helps in back crosses and in obtaining desirable recombinations (Abdelnoor et al., 1995).

Genetic diversity can be evaluated by prediction techniques or by the quantification of the heterosis shown by the hybrids (Cruz and Regazzi, 1997). However, the latter can be obtained only if the cultivars are crossed, which is unnecessary when prediction techniques are used. In the case of many cultivars with useful traits, the large number of crosses needed hinders the use of the hybrid quantification technique, especially for self pollinating species such as soybean. Also, although genetic diversity is a necessary condition for heterosis, it is not enough to guarantee it, as heterosis depends on the differences in allele frequencies and the dominance of the loci involved (Cress, 1966).

Genetic diversity has been studied using different data, such as agronomic traits (Engels, 1983; Bekele et al., 1994), the parentage coefficient (Hiromoto and Vello, 1986; Vello et al., 1988), enzymes (Cox et al., 1985b), molecular markers RFLP (Restriction

Fragment Length Polymorphisms) (Keim et al., 1989), RAPD (Random Amplified Polymorphic DNA) (Abdelnoor et al., 1995), AFLP (Amplified Fragment Length Polymorphism) (Maughn et al., 1996), and SSR (Micro satellites or Simple Sequence Repeats) (Taramino and Tingey, 1996).

The varions techniques used to evaluate genetic diversity have different limitations and advantages (Patterson et al., 1991; Gizlice et al., 1993b). The parentage coefficient measures similarity by descent, neither considering the total genetic similarity of the individuals (Bered et al., 1997) nor measuring the relationship among the genetic backgrounds (Gizlice et al. 1993b). On the other hand, it does not require crossing to evaluate genetic dissimilarity. Molecular markers used in analyses may not be distributed in the linkage groups (Gizlice et al., 1993b). They have, however, the advantage of producing many markers for genetic characterization by quick and simple procedures.

Agronomic diversity evaluation based on agronomic traits of interest has coincided with genetic divergence, being a function of the number of markers, the association with loci for quantitative traits ("quantitative traits loci - QTL") and the parentage coefficient (Gizlice et al., 1993a). The agronomic reliability of the genetic distance estimates based on molecular and genealogical data may be evaluated by their correlation with the variance of their progeny (Gizlice et al., 1993b)

Studies on genetic diversity in elite soybean cultivars have shown low variability, regardless of whether the data are of molecular or agronomic origin (Delannay et al. 1983; Cox et al., 1985a; Hiromoto and Vello, 1986; Keim et al., 1989; Sneller, 1994; Abdelnoor et al., 1995; Sneller et al., 1997).

Multivariate techniques have contributed to the estimation of genetic diversity among populations of several crops, to the selection of cultivars for hybridization and, to eliminate double accessions in germplasm banks, when a set of important traits is considered simultaneously Cruz (1990). The principal component analysis (multivariate technique) was used in the selection of phenotypic traits for soybean cultivar differentiation (Sediyama et al., 1989). The same analysis was applied to information obtained by molecular markers (RAPD) to differentiate among 38 soybean cultivars (Abdelnoor et al., 1995). These authors identified two genetically divergent groups, A and B, and five subgroups, two belonging to group A and three to group B. Four of these 38 cultivars, IAC-12, FT-Cristalina, Doko RC and UFV-10 were classified in genetically divergent groups. These cultivars, however, although divergent by RAPD, were considered to have a narrow genetic base due to their genealogy and enzymes study (Oliveira, 1992).

The present study was carried out to assess and compare the genetic diversity evaluated by prediction techniques using agronomic traits and genealogical inference of elite soybean cultivars with narrow genetic base. The genetic diversity previously assessed by molecular markers was also compared to that obtained from agronomic traits.

MATERIAL AND METHODS

Four soybean (Glycine max. (L.) Merrill) cultivars, selected by their genetic diversity assessed by RAPD (Random Amplified Polymorphic DNA) molecular markers (Abdelnoor et al., 1995), were crossed. The cultivar groups and sub-groups established by the Abdelnoor et al. (1995) molecular analysis and their genealogy are as follows (Figure 1): UFV-10: Santa Rosa (D49-772 x LA 41-1219) x UFV-1, belonging to group A, in the RAPD diversity analysis; IAC-12: Paraná x IAC 73-231 cross, belonging to group B1, in the RAPD diversity analysis; Doko RC: RB72-1 population, formed by a pool of seeds from the

following crosses: [E70-46 x Viçoja (UFV-1 parent)], (E70-47 x Viçoja), (Hill x E70-74), (E70-46 x Pickett), (E70-47 x F65-1376) and (Davis x IAC 79-308), belonging to group B2 in the RAPD diversity analysis and FT-Cristalina, natural cross of an unknown cultivar with UFV-1, belonging to Group B3 in the RAPD markers diversity analysis. Hiromoto and Vello (1986) suggested the probable genealogies to Doko: Viçoja x (Hill x PI 240664) and FT-Cristalina: Davis x UFV-1.

The F_1 progenies and the cultivars were assessed in a glass house environment, under controlled humidity and temperature, in a completely randomized design with ten treatments (six hybrid combinations and four parents) and four replications. The temperature was kept at 26°C and 22°C for day and night, respectively. The relative air humidity was approximately 70%. The experimental plot consisted of a single pot with two plants. The traits were assessed in each individual plant.

The following traits were assessed: number of days to flowering (NDF), counted from germination to the opening of one flower on the main stem; plant height at flowering (PHM), measured in centimeters, from soil level to the apex of the main stem, as the first flower opened; number of nodes at flowering (NNF) counted on the main stem, from the unifoliolate leaf node as the first flower opened; number of secondary branches at flowering (NSB); height of the first pod at maturity (HFP) measured in centimeters from soil level to the main stem pod insertion closest to the soil; plant height at maturity (PHM) measured in centimeters from soil level to the insertion of the last pod; number of nodes at maturity (NNM) counted on the main stem, from the unifoliolate leaf node; number of secondary branches at maturity (NSBM); number of pods with seeds on the main stem (NPS); number of pods with seeds on the secondary branches (NPSB); number of pods with seeds per plant (NPP); number of seeds per plant (NSP); mean number of seeds per pod (NMSP) obtained by the quotient between NPP and NSP; weight of 100 seeds (WHS) given by the ratio between the total seed weight and the total number of seeds, multiplied by 100; number of days to maturity (NDM), counted from germination to when the plant had 90% of ripe pods; yield per plant (PRO) assessed in grams, based on total seed weight of each plant.

The parentage coefficient estimates and the Mahalanobis' generalized distance obtained from the multivariate analysis of the assessed traits, as described by Rao (1952) and Cruz and Regazzi (1997), were used as the prediction statistics to assess the genetic diversity among the pairs of cultivars. A group analysis was carried out using Tocher's optimization method, based on the Mahalanobis generalized distance matrix.

The parentage coefficients were estimated using the identity by descent method developed by Malècot (1948) and calculated according to Falconer (1981). A coefficient equal to zero and to one indicates absence and closest parentage relationship between two individuals, respectively. An analysis of variance was carried to validate the prediction information on the genetic diversity for the agronomically important traits NDF, NDM, HFP, PHM, NPP, WHS, NSP, and PRO.

The presence of significant genetic variability among treatment, cultivars and progenies was assessed by the F test. The statistical and genetic analyses were performed using the GENES program (Cruz, 1997) developed by the Genetic Sector of the Department of General Biology at the Federal University of Viçosa and the SAEG (Statistical Analysis System), also developed at the Federal University of Viçosa.

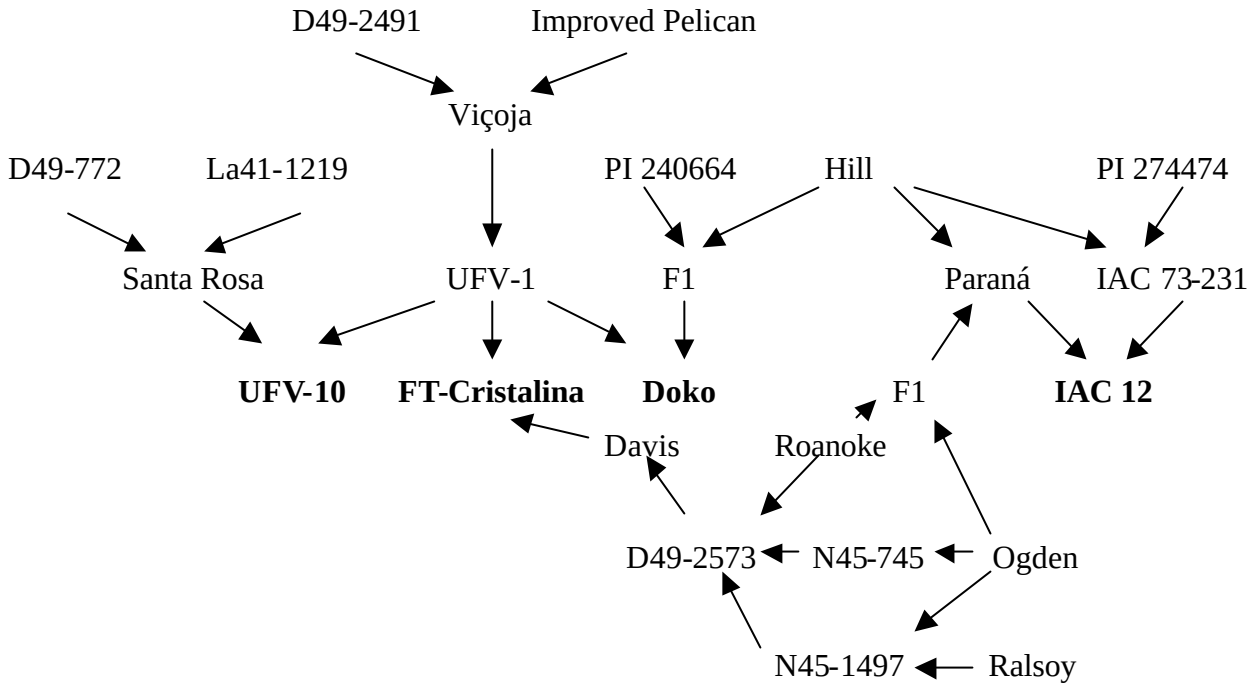

Figure 1 - Pedigree relationships of soybean cultivars.

RESULTS AND DISCUSSION

Analyses of variance were used to assess the genetic diversity among treatments, cultivars and progenies obtained by crossing. The significance of these sources of variation was checked by the F test (a posteriori check). Table 1 shows the analysis of variance of the means of eight economically important traits. The treatment effect and its partition into cultivars and progeny (F_1) effects, were significant for all the traits, except PHM, indicating the existence of agronomic divergence among cultivars and progenies ($P< 0.01$). The contrast between cultivars and F_1 (C vs. F_1) was only significant for NDF, indicating average heterosis. So, this elite soybean cultivars show agronomic divergence in economically important traits and any cross between cultivars are possible, although the genetic base of the cultivars is narrow. Brazilian soybean cultivars showed the same narrow base as the American germplasm, since the Brazilian germplasm was formed by lines introduced from the United States and eleven such ancestors collectively represent 89% of the Brazilian gene pool (Hiromoto and Vello, 1986). Abdelnoor et al. (1995) using molecular markers (RAPD) confirmed the narrow genetic base of the Brazilian soybean germplasm. Rasmusson and Phillips (1997) suggested that elite gene pools have inherent mechanisms to provide a continuing source of new genetic variability because in plant breeding programs and long-term selection experiments in several organisms the genomes were more plastic and amenable to selection than previously assumed.

For each cross, the average of the generations is 1/2 (P1 + P2) = m + [i], were P1 and P2 are parents; m represent mean between the two homozygotes; and, [i] represent homozygote x homozygote interaction. The F_1 might not be efficient in identifying the cross which will give the best homozygous lines of the advanced generations (Fa = m) due to the heterosis effect because F_1 = m + [h], were [h] represent the departure in phenotype of the heterozygote the mid-point between homozygotes or heterozygote effect for all loci. (Mather and Jinks, 1982) . However, in this study, the non-significance of the contrast for the majority of traits indicated that heterosis may not be present.

Table 1 – Summaries of the analysis of variance of the number of days to flowering (NDF), number of days to maturity (NDM), height of first pod at maturity (HFP), plant height at maturity (PHM), number of pods with seeds formed per plant (NPP), weight of 100 seeds (WHS), number of seeds per plant (NSP), yield per plant (PRO) in soybean cultivars and their progenies.

		NDF	NDM	HFP	PHM	NPP	WHS	NSP	PRO
Treatments	(9)	97**	201**	13**	89**	826 **	6.94 **	3853 **	58.82 **
Cultivar(C)	3	158**	428**	16**	215**	888**	10.20**	4383**	85.00**
Progeny (F_1)	5	47**	105**	15**	29ns	950**	6.22**	3992**	51.15**
C vs. F_1	1	159**	1.56ns	0.1ns	8.98ns	16ns	0.76ns	1563ns	18.63ns
Error	30	4.62	6.57	3.90	23.97	189.8	2.26	810	14.17
CV (%)		4.7	2.3	13.1	6.34	15.45	9.3	16.4	13.64

**Significant at the 1% level of probability F test; Ns - Non significant.

The Malècot parentage coefficient and relationship between cultivars are shows in Table 2 and Figure 1. FT-Cristalina, Doko RC and UFV-10 have at least the UFV-1 variety as the common parent in their genealogies and, therefore, a parentage coefficient equal or greater than 0.25. UFV-1 is a selection in Viçoja and the genetic distance between these cultivars is only two percents using RAPD markers (Abdelnoor et al., 1995). The parentage coefficient between FT-Cristalina and Doko is 0.277 (Hiromoto and Vello, 1986). IAC-12 does not have a common ancestor with UFV-10 and show zero parentage coefficient. The genetic relationship between IAC-12/ FT-Cristalina is through Roanoke and Ogden with parentage coefficient of 0.09375. The parentage coefficient between Doko and IAC-12 is 0.125. The parentage coefficient between Roanoke and Davis is 0.263 (Gislize et al., 1996). The four studied cultivars can be placed into two groups according to their genealogy (Table 3). The first group is formed by the IAC-12 variety. The second is formed by FT-Cristalina, Doko RC, and UFV-10. Gizlice et al. (1993a) stated that to maintain genetic diversity in American soybean germplasm it would suffice to cross cultivars whose parentage coefficient was less than 0.25. According to this concept, the crosses between UFV-10, Doko RC and FT-Cristalina have a tendency to narrow the genetic base. Therefore, the suitable biparental crosses, based exclusively on the prediction data from the genealogy, should be made among groups that is, between IAC-12 and UFV-10, Doko RC and FT-Cristalina. This analyses shows that FT-Cristalina, Doko RC and UFV-10 cultivars are genetically similar. Abdelnoor et al. (1995), however, showed that the cultivars used in this study belong to genetically different subgroups (Table 3). The authors reported that for greater genetic diversity crosses should be made among the cultivars belonging to different subgroups, which showed greater dissimilarity. The groups formed by genealogical inference differed from those obtained by molecular markers.

Table 2 shows the genetic distances among pairs of cultivars measured by Mahalanobis D^2 statistic, using the 16 assessed agronomic traits. They indicated that the closest pair is FT-Cristalina/Doko RC and the most distant pairs are IAC-12/ UFV-10 and IAC-12/ Doko RC. Thus, the suitable biparental crosses, based

exclusively on prediction data from D_2, should be made between IAC-12/ UFV-10 and IAC-12/Doko RC. Generally, D^2 among non-related individuals was greater than among related individuals grouped according genealogical inference, except to FT-Cristalina/IAC-12. The genetic divergence evaluated by prediction techniques using agronomic traits of these parents are partially coincident with those suggested by the genealogical inference statistics. However, in the study by Abdelnoor et al. (1995), the ascending order of dissimilarity among pairs of cultivars obtained by molecular markers is the following (Table 2): FT –Cristalina and UFV-10, IAC-12 and UFV-10, IAC-12 and Doko RC, FT-Cristalina and IAC-12, UFV-10 and Doko RC, FT-Cristalina and Doko RC. Therefore, the orders of dissimilarity among the cultivar pairs given by Mahalanobis D2 and by molecular data are very different, but both show that the cultivars are genetically divergent.

Table 2 – Dissimilarity among soybean cultivars for 16 traits, based on Mahalanobis generalized distance ($D^2_{ii'}$), Málecot parentage coefficient (between parenthesis) (both above the diagonal) and pairwise genetic distances (%) (below the diagonal) from Abdelnoor et al. (1995)

	UFV-10	Doko RC	FT-Cristalina	IAC-12
UFV-10		201 (0.25)	204 (0.25)	554 (0.0)
Doko RC	22		99 (0.277)	354 (0.125)
FT-Cristalina	15	24		180 (0.09375)
IAC- 12	19	20	21	

Tocher's optimization method formed four heterotic groups among the cultivars (Table 3). Group 1 is made up of the IAC-12 cultivar. Group II is formed by the FT-Cristalina cultivar. Group III is formed by the Doko RC cultivar and group IV by the UFV-10 cultivar. The grouping statistics indicated that the cultivars are genetically divergent, and formed four different groups similar to those given by the molecular data using RAPD markers obtained by Abdelnoor et al. (1995). Crosses between any cultivars are indicated. The groups formed by Tocher's method disagree with the results of the prediction analyses base on the cultivar genealogy.

Table 3 – Grouping of soybean cultivars by genealogical inference, Tocher's optimization method, RAPD markers (ABDELNOOR et al., 1995) and isozyme peroxidase systems extracted separately from the seed coat and leaf (Oliveira,1992).

Groups	Genealogical Inference	RAPD markers	Tocher	Peroxidase seed and leaf isozyme	Peroxidase root isozyme
I	IAC-12	IAC-12	IAC-12	IAC-12	IAC-12
II	FT-Cristalina Doko RC UFV-10	FT-Cristalina	FT-Cristalina	FT-Cristalina Doko RC UFV-10	FT-Cristalina
III	-	Doko RC	Doko RC	-	Doko RC UFV-10
IV	-	UFV-10	UFV-10	-	-

Several dissimilarity groups were obtained by Oliveira (1992) who used five isozyme systems to characterize 14 cultivars, among them Doko (Doko RC recurrent cultivar), FT-Cristalina, IAC-12 and UFV-10, which were all used in the present study. The author classified the cultivars in two groups, according to the electrophoresis phenotypes of peroxidase extracted separately from the seed coat and leaf. One group was formed by IAC-12 and another by FT-Cristalina, Doko and UFV-10 (Table 3). This grouping is close to that obtained by genealogical inference in this study. On the other hand, tree groups were obtained when the electrophoresis phenotypes from peroxidase extracted from the root were analyzed: one formed by UFV-10 and Doko, and another two formed by IAC-12 and FT-Cristalina. Therefore, the results found by Oliveira (1992) are generally in agreement with those obtained in this study (Tocher) and those of Abdelnoor et al. (1995), who discriminated the cultivars in two or more groups.

The different classification techniques of the same genotypes should produce similar results if these classifications are to reflect the true association among the genotypes (Mumm et, al., 1994). In this study, however, it should be considered that the limitations of genealogical inference and molecular markers could be affecting the results. Thus, the groups of genetic dissimilarities previously obtained by each technique using the parents agronomic traits and biotechnology pointed out to the presence of genetic variability among the cultivars. The partition of cultivars in groups is artificial, so different criterions produce different groups, but every technique was efficient to separate the cultivars with maximum genetic divergence. This is important to optimize the choice of parents for crosses having specific objectives, such as populations with large genetic variability and commercial viability.

CONCLUSIONS

The prediction techniques and the analysis of variance of the progenies indicated the presence of genetic diversity among the soybean cultivars with narrow genetic base;

The cultivar groups formed by genealogical inference were different from those obtained previously (Tocher);

Genetic divergence evaluated by the application of prediction techniques usin g agronomic traits of the parents was similar with the agronomic divergence of the progenies;

The all prediction techniques are similar to separate only soybean cultivars with maximum genetic divergence.

It's possible selecting soybean cultivars with genetic divergence among elite cultivars.

The crosses among soybean cultivars that maximize the genetic divergence were different by prediction techniques.

RESUMO

Divergência Genética Entre Cultivares Elites De Soja Com Base Genética Estreita

Os objetivos deste trabalho foram avaliar e comparar a diversidade genética entre cultivares elites de soja com base genética estreita usando técnicas preditivas a partir de características agronômicas e inferência genealógica. Estas predições foram comparadas com a diversidade agronômica observada nas progênies. Para isso, avaliou-se os cultivares FT-Cristalina, Doko RC, IAC-12, UFV-10, selecionadas com base na diversidade genética obtida a partir de marcadores moleculares. As seis progênies (F1) e os quatro cultivares foram avaliados em casa-de-vegetação com controle de umidade e temperatura, em delineamento experimental inteiramente casualizado com quatro repetições. A diversidade genética foi avaliada de forma preditiva pela inferência genealógica e pela análise de agrupamento com base nas

características agronômicas utilizando a distância generalizada de Mahalanobis e o método de otimização de Tocher. Todas as técnicas de predição e a análise de variância das progênies indicaram a presença de diversidade genética entre os cultivares com base genética estreita. Os grupos de cultivares formados pela inferência genealógica foram diferentes daqueles obtidos previamente pelo método de otimização de Tocher. A divergência genética constatada pela aplicação das técnicas de predição dos pais foi similar com a divergência agronômica das progênies. Todas as técnicas de predição foram similares para separar cultivares com a máxima divergência genética. É possível selecionar cultivares de soja com divergência genética entre cultivares elites. Os cruzamentos entre cultivares de soja que maximizam a divergência genética foram diferentes de acordo com as técnicas de predição.

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