GENETIC SIMILARITY REVEALED WITH RAPD AND DAF MARKERS IN SOME MAIZE INBREDS

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ABSTRACT

Knowledge of the genetic relationship among breeding materials could help to avoid the risk for an increasing uniformity in the elite germplasm and ensure long-term selection gains (Messmer et al., 1993). Molecular markers allow a direct comparison of the similarity of genotypes at the DNA level, such as RAPD and DAF, developed in our laboratory at Fundulea (Iuora°, 1999). The plant material provided by breeding maize laboratory consisted in two heterotic groups of inbreds, related among them within each group. A number of 42 decamer primers were used in DNA-PCR amplification. Genetic similarity was calculated according to the formula proposed by Nei and Li in 1979 (Lübberstedt et al., 2000). Genetic similarity coefficient revealed a relationship among genotypes within each group. The increased number of bands in DAF patterns suggests that DAF analysis is more informative than RAPD analysis. The unique RAPD and/or DAF bands confirmed by two different PCR amplifications, or the lack of consistent bands comparative with other genotypes within the group could facilitate the selection of genotypes carrying new and valuable traits as a result of genetic recombination.

Key words: molecular markers, maize inbreds

INTRODUCTION

Back-cross populations or F_2 generations are often used, as base materials for germplasm recycling (Darrah and Zuber, 1986). If the breeding potential of such populations could be predicted in advance from the traits of parental lines, this would increase the potential and efficiency of breeding programmes and consequently it would allow the concentration of efforts on the most promising populations.

In the last 40-50 years, a large number of maize inbreds have been developed from a limited number of elite lines and elite line synthetics. Knowledge of the genetic relationship among breeding materials should avoid the increasing of uniformity in the elite germplasm and could ensure long-term selection gains (Messmer et al., 1993)

Molecular markers allow a direct comparison of the similarity of genotypes at the DNA level. The RFLPs for instance, have been used with the major advantage of finding a large number of polymorphic loci in breeding materials. This permits to define heterotic groups, assign inbred lines to such groups, reveal genetic relationships among lines and identify germplasm sources (Messmer et al., 1992).

Molecular markers based on polymerase chain reaction (PCR) as RAPD, AFLP, AP-PCR, SSR, DAF are being used as important tools for the breeders (Lübberstedt et al., 2000; Caetano-Anollés et al., 1991; Hahn et al., 1995; Jones et al., 1997). AFLP and SSR markers generate multiple bands in a single assay.

The objectives of this study were to evaluate genetic similarity determined by RAPD and DAF within two groups of maize inbreds. Genetic diversity estimation of inbreds for heterosis potential should be of a great interest for breeding (Becker and Link, 2000).

MATERIALS AND METHODS

Plant material consisted of two groups of inbreds, related within each group: 28058, F 114, F 316 and Mo 17, F 730, F 736, F 213, F 210, F 644, F 911, F 1109, developed by maize breeding department at Fundulea Institute.

DNA extractions were performed following a protocol based on CTAB, on embryos excised from the seeds. PCR DNA amplifications were achieved in a M.J. Research Thermocycler with 36 cycles as 95°-55°-72° and a final extension at 72° for 7 minutes (Iuora°, 1999).

A number of 42 different primers have been tested (UBC and Operon Technology). PCR products were evaluated by RAPD analysis (ethidium bromide staining and UV bioprint-

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ing) and DAF analysis (silver staining) as described previously.

Genetic similarity coefficients were calculated according to the formula:

$$GS= 2Nij/ Ni + Nj$$

were Nij is the number of bands in common between line i and j, and Ni and Nj are the total number of bands in the lines. Mean GS of lines i to a set J of inbreds was obtained by averaging individual GS estimates, according to the following formula:

$$Mean\ GSiJ = \Sigma Gsij/J$$

were J is the number of elements in set J (Lübberstedt et al., 2000).

RESULTS AND DISCUSSIONS

The inbreds F 114 and F 316 related to 28058 germplasm were tested with 42 different decamers primers. Genetic similarity for the both pairs of inbreds was calculated based on RAPD bands analysis in agarose gels and DAF bands analysis in polyacrilamide gels (Figures 1 and 2).

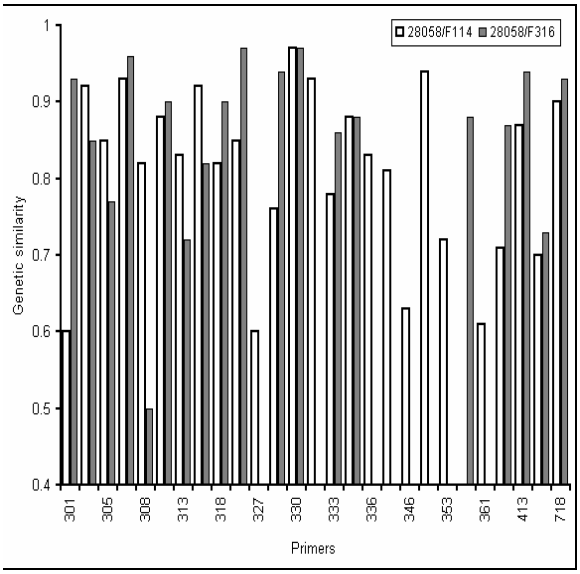

Figure 1 . Genetic similarity for two paires of related genotypes obtained by RAPD analysis

Genetic similarity coefficients (GS), computed from RAPD analysis (averaged over 25 primers), between 28058 germplasm and two inbred lines related to 28058 are presented in Table 1a. The two inbreds, F114 and F316, were extracted from two different F_2 populations derived from two single crosses between two different inbreds and 28058 germplasm, so that the co-ancestry of F114 and F316 was theoretically 50% provided by 28058 germplasm.

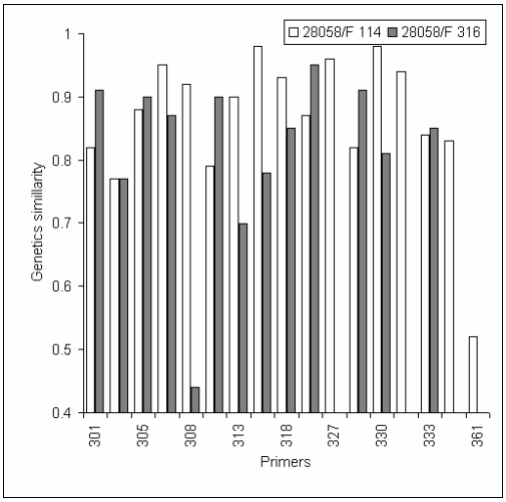

Figure 2 . Genetic similarity of two paires of related genotypes obtained by DAF analysis

Table 1a: Genetic similarity and mean genetic similarity estimates, calculated from RAPD (averaged over 25 primers) data of two maize inbreds related to elite 28058 germplasm

Genotypes	%	28058	F 114	F 316	Mean GS
28058	100		0.79	0.85	0.82
F 114	50			0.70	0.74
F 316	50				0.77
Average					0.78

Genetic similarity coefficient computed between the two lines and 28058 germplasm was however larger than 0.50, being 0.79 for F 114 and 0.85 for F 316, suggesting a stronger degree of relationship between the two lines on the side of their co-ancestry coming from the 28058 germplasm. Consequently, the GS coefficient between the two lines had, also, a high value of 0.70, although the two inbred lines crossed with 28058 to produce the F_2 populations were completely different and unrelated. General mean of GS coefficient was 0.78. Similar results were obtained when GS coefficients were computed from DAF analysis with 17 primers, but the values were even more larger than those produced by RAPD analysis (Table 1b) (general mean of GS coefficient 0.81).

Table 1b: Genetic similarity and mean genetic similarity estimates, calculated from DAF (averaged over 17 primers) data of two maize inbreds related to elite 28058 germplasm

Genotypes	%	28058	F 114	F 316	Mean GS
28058	100		0.86	0.81	0.83
F 114	50			0.75	0.80
F 316	50				0.78
Average					0.81

Even if the number of consistent polymorphic bands was low, in the case of this group of related lines, that could be extremely important for germplasm diversity, as genetic recombination released through selfing and selection.

The results obtained from RAPD analysis with the primer UBC 681 for the inbred line Mo 17 and other seven inbred lines selected from F_2 populations originating from crosses between Mo 17 and seven different inbred lines, presented in table 2a, show, as expected, a relative high degree of relationship among all inbreds (mean GS coefficient 0.62). A large genetic similarity (mean GS = 0.72) was registered between Mo 17 and the other seven related lines, with small variation from GS = 0.86 (with F 644) to 0.55 (with F 911). The inbred line F 644 gave on the average with all the other inbreds from this group a mean GS coefficient of 0.78, but with important variations from 0.96 (with F 210) to 0.46 (with F 911). The other inbred lines produced smaller GS coefficients and larger variations. The lowest GS coefficients were obtained for the pairs F 911/F 213 (0.29), F 210/ F213 (0.21), F 730/F 210 (0.34), F 210/F 1109 and F 730/F 210 (0.34). Data suggest that F210 and F911 are on an average the most diverse from the other lines, with mean coefficients of 0.47 and 0.48 respectively, followed by F 213 with a mean GS coefficient of 0.57.

DAF analysis with the same primer, UBC 681, for the same group of inbred lines, presented in Table 2b, resulted in a slightly smaller overall mean of GS coefficient of 0.52 as compared to 0.62 obtained with RAPD.

The estimated relationship between Mo17 and the derived lines was smaller, the mean GS coefficient being 0.60. On an average, the most genetically diverse inbreds seemed to be in this case F 1109 (GS = 0.37),

Table 2a. GS and mean GS of 7 pairs of maize inbreds, by RAPD analysis with primer UBC 681

Genotypes	Mo 17	F 644	F 730	F 736	F 911	F 213	F 210	F 1109	Mean GS
Mo 17		*20/17**	*20/19*	*20/16*	*20/9*	*20/18*	*20/10*	*20/14*	
		17/4**	15/2	14/2	8/1	14/2	9/0	11/2	
		0.86* **	**0.77**	**0.77**	**0.55**	**0.73**	**0.60**	**0.74**	**0.72**
F 644	**0.86**		*17/19*	*17/16*	*17/9*	*7/18*	*17/10*	*17/14*	
			14/3	13/3	6/3	13/4	13/4	13/1	
			0.82	**0.78**	**0.46**	**0.74**	**0.96**	**0.83**	**0.78**
F 730	**0.77**	**0.82**		*19/16*	*19/9*	*19/18*	*19/10*	*19/14*	
				13/3	7/2	14/4	5/5	10/4	
				0.74	**0.50**	**0.73**	**0.34**	**0.60**	**0.64**
F 736	**0.77**	**0.78**	**0.74**		*16/9*	*16/18*	*16/10*	*16/14*	
					8/1	12/4	4/6	12/2	
					0.64	**0.70**	**0.30**	**0.80**	**0.68**
F 911	**0.55**	**0.46**	**0.50**	**0.64**		*9/18*	*9/10*	*9/14*	
						4/5	5/4	5/4	
						0.29	**0.52**	**0.43**	**0.48**
F 213	**0.73**	**0.74**	**0.73**	**0.70**	**0.29**		*18/10*	*18/14*	
							3/7	10/4	
							0.21	**0.62**	**0.57**
F 210	**0.60**	**0.96**	**0.34**	**0.30**	**0.52**	**0.21**		*10/14*	
								5/5	
								0.34	**0.47**
F 1109	**0.74**	**0.83**	**0.60**	**0.80**	**0.43**	**0.62**	**0.34**		**0.62**
Average									0.62

*20/17 = *total no. of bands for the two inbreds*
**17/4 = no. of common bands/ polymorphic bands
***0.86 = GS= genetic similarity

Table 2b. GS and mean GS of 7 paires of maize inbreds by DAF analysis with primer UBC 681

Genotypes	Mo 17	F 644	F 730	F 736	F 911	F 213	F 210	F 1109	Mean GS
Mo 17		*26/28**	*26/23*	*26/26*	*26/19*	*26/33*	*26/30*	*26/6*	
		19/4**	18/4	16/6	10/2	19/5	16/6	6/0	
		0.70* **	**0.73**	**0.59**	**0.44**	**0.77**	**0.57**	**0.37**	**0.60**
F 644	**0.70**		*28/23*	*28/26*	*28/19*	*28/33*	*28/30*	*28/6*	
			18/5	15/5	10/3	23/3	15/5	6/0	
			0.70	**0.55**	**0.42**	**0.75**	**0.51**	**0.35**	**0.57**
F 730	**0.73**	**0.70**		*23/26*	*23/19*	*23/33*	*23/30*	*23/61*	
				16/5	11/3	17/5	13/6	6/0	
				0.54	**0.52**	**0.60**	**0.49**	**0.41**	**0.57**
F 736	**0.59**	**0.55**	**0.54**		*26/19*	*26/33*	*26/30*	*26/6*	
					13/2	19/6	16/7	6/0	
					0.57	**0.64**	**0.57**	**0.37**	**0.55**
F 911	**0.44**	**0.42**	**0.52**	**0.57**		*19/33*	*19/30*	*19/6*	
						9/8	10/7	6/0	
						0.34	**0.40**	**0.48**	**0.45**
F 213	**0.77**	**0.75**	**0.60**	**0.64**	**0.34**		*33/30*	*33/6*	
							16/9	6/0	
							0.48	**0.30**	**0.55**
F 210	**0.57**	**0.51**	**0.49**	**0.57**	**0.40**	**0.48**		*30/6*	
								6/0	
								0.33	**0.48**
F1109	**0.37**	**0.35**	**0.41**	**0.37**	**0.48**	**0.30**	**0.33**		**0.37**
Average									**0.52**

*26/28 = *total no. of bands for the two inbreds*
**19/4 = no. of common bands/ polymorphic bands
***0.70 = GS= genetic similarity**

F 911 (GS =0.45) and F 213 (GS = 0.55). Several pairs of lines produced lower GS coefficients suggesting a larger genetic diversity (F 911/F 213, F 213/F 210, F 210/F 1109, F 730/F 210 and F 730/F 1109).

Data obtained with DAF analysis are generally consistent with those generated by RAPD. The differences in GS coefficients among inbred lines, although all the lines have in their co-ancestry a common germplasm source (Mo 17), could be explained by the segregation caused through selfing and selection effects which kept or eliminated some segregates on the basis of their economic value and combining ability.

The higher number of DAF bands as compared with RAPD suggests that DAF analysis could have a more informative potential than RAPD.

CONCLUSIONS

The two types of markers, RAPD and DAF, estimated the genetic similarity degree among related maize inbred lines belonging to two groups of germplasm in a similar way, the differences observed being relatively small and without a major importance in discriminating the two types of markers for their efficiency in appreciating the genetic diversity of the inbreds. It seems that DAF, due to a larger number of bands has a higher potential in differentiating the genotypes in some instances.

Since relative similar results were obtained by using a single primer or a larger number of primers, it seems that in some instances the utilization of a large number of primers is not mandatory or do not contribute

significantly to the improvement of data. However, the utilization of a large number of primers is recommended for a better coverage of the whole genotype, and for a more precise estimation of the genetic diversity of the inbreds.

Data obtained suggest that where rigorous selection in any group, within different segregating but strongly related populations, the degree of genetic similarity of the progenies extracted from those populations could be significantly different from that expected in accordance with the co-ancestry of the progenies.

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Table 1. Influence of aluminum ions, in reaction mixture, on the level of saccharasic activity in a reddish-brown soil fertilized with compost with different quantities (glucose+fructose-mg/100 g soil dw/24 hours)

A- Factor	B – Factor – COMPOST (t/ha)								Average (A)	
	b1-0	%	b2-0	%	b3-0	%	b4-0	%		%
a1–without Al^{3+}	b 3287	100	b 4028	100	b 2579	100	b 3472	100	b 3341	100
a2- with Al^{3+}	a 4228	129	a 5019	125	a 3472	135	a 4528	130	a 4312	129
Average (B)	3757 c		4523 a		3025 d		4000 b			

LD P	5%	1%	0,1%
A	291	673*	2143
B	101	142	201*
AB	302	628*	1799
BA	144*	201	284

Table 2. Influence of aluminum ions, in reaction mixture, on the level of saccharasic activity in a chernozem mineral fertilized or manured with farmyard compost (glucose+fructose-mg/100 g soil dw/24 hours)

A- Factor	B – Factor – COMPOST (t/ha)										Average (A)	
	b1-0	%	b2-$N_{32}P_{32}$	%	b3-$N_{94}P_{96}$	%	b4-$N_{128}P_{128}$	%	b5 compost	%		%
a1–without Al^{3+}	b 1564	100	b 1496	100	b 1459	100	b 1401	100	b 1732	100	b 1530	100
a2- with Al^{3+}	a 1686	108	a 1581	106	a 1684	115	a 1589	113	a 1864	108	a 1681	110
Average (B)	1625 b		1538 d		1571 c		1495 e		1798 a			

LD P	5%	1%	0,1%
A	7	17	54*
B	14	20	27*
AB	19	28	45*
BA	20*	28	39