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Genetic relationships among melon breeding lines revealed by RAPD markers and agronomic traits

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Abstract RAPD markers and agronomic traits were used to determine the genetic relationships among 32 breeding lines of melon belonging to seven varietal types. Most of the breeding lines were Galia and Piel de Sapo genotypes, which are currently being used in breeding programmes to develop new hybrid combinations. A total of 115 polymorphic reliable bands from 43 primers and 24 agronomic traits were scored for genetic distance calculations and cluster analysis. A high concordance between RAPDs and agronomic traits was observed when genetic relationships among lines were assessed. In addition, RAPD data were highly correlated with the pedigree information already known for the lines and revealed the existence of two clusters for each varietal type that comprised the lines sharing similar agronomic features. These groupings were consistent with the development of breeding programmes trying to generate two separate sets of parental lines for hybrid production. Nevertheless, the performance of certain hybrids indicated that RAPDs were more suitable markers than agronomic traits in predicting genetic distance among the breeding lines analysed. The employment of RAPDs as molecular markers both in germplasm management and improvement, as well as in the selection of parental lines for the development of new hybrid combinations, is discussed.

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Genetic diversity ' Agronomic markers ·
Germplasm management

Introduction

The assessment of the genetic variability existing in the germplasm of different species is of interest not only in the organization and conservation of genetic resources, but also for practical applications such as broadening of the genetic base of species and exploitation of heterosis. Increasing the genetic base is a major concern in species where inbreeding practices have resulted in the loss of genetic diversity (Graham et al. 1996), a process that could be responsible for the unsuccessful development of new combinations. On the other hand, since hybrid heterosis seems to be related to the genetic divergence of parental lines (Lee et al. 1989; Smith et al. 1990; Sekhon and Gupta 1995), information on the genetic similarity between genotypes of agronomic importance species may also facilitate the prediction of crosses that will produce hybrids with higher performance.

The relative genetic diversity among individuals or populations can be determined using morphological and molecular markers. Phenotypic characters have a limited importance since they are generally influenced by environmental factors and developmental stage of the plant and because in some species adequate levels of phenotypic polymorphism are not available (Tatineni et al. 1996). On the contrary, molecular markers, based on DNA sequence polymorphism, are independent of environmental conditions and show higher levels of polymorphism. Several methods such as isozymes or restriction fragment length polymorphisms (RFLPs) have been used in the analysis of genetic relationships in different species (Liu and Furnier 1993; Sekhon and Gupta 1995; Tsegaye et al. 1996). More recently

randomly amplified polymorphic DNAs (RAPDs) have been widely employed for their simplicity and their capacity to detect genetic variation among very closely related genotypes in a number of species like *Brassica* (Jain et al. 1994), *Lens* (Abo-elwafa et al. 1995), *Petunia* (Cerny et al. 1996) or *Pisum* (Hoey et al. 1996). At the moment, other molecular markers such as amplified fragment length polymorphisms (AFLPs) or simple sequence repeats (SSRs) are being used because they seem to detect higher level of polymorphism (Yang et al. 1994; Rongwen et al. 1995; Sharma et al. 1996, Katzir et al. 1996). However, these latter two also have some disadvantages compared to RAPDs, such as the need for radioactive labelling or previous sequence information, which limit their potential applications.

A wide variety of fruit characteristics has allowed botanists to classify melon into ten varietal types (Whitaker and Davis 1962), among which '*cantalupensis*','*reticulatus*', '*inodorus*' and '*saccharinus*' are mainly cultivated in Spain. However, this high morphological variability has not been reflected at the molecular level. Isozyme variability has been found to be very low in this species (Dane 1983; Perl-Treves et al. 1985; Staub et al. 1987), while later studies with RFLPs detected enough polymorphism to enable the different groups within the germplasm of cultivated melon to be discriminated and classified (Neuhausen 1992). Unfortunately, discrimination among lines belonging to the same varietal type is not as effective. The high level of repetitive DNA and the low level of DNA variation in unique sequences could explain this paucity of RFLPs markers in melon (Shattuck-Eidens et al. 1990; Neuhausen 1992).

RAPDs have been demonstrated to be useful for the identification of markers linked to traits of agronomic importance; for example fusarium wilt resistance in melon (*Fom* 2 gene; Wechter et al. 1995). Together with isozymes and RFLPs, 64 RAPD markers have recently been placed in the genetic map of melon (Baudracco-Arnas and Pitrat 1996). In this map, RAPDs are evenly distributed throughout the melon genome, as are RFLPs, indicating that both types of markers are equally applicable for melon genome analysis. RAPDs have also allowed the detection of polymorphism among eight different varietal types of melon (Katzir et al. 1996). Nevertheless there is no information regarding the suitability of RAPDs to analyse the relationships among closely related lines which had been well-characterized agronomically and are being employed in breeding programmes of melon.

We report here the use of RAPD markers to determine the genetic relationships not only among different varietal types of melon but also among breeding lines within two varietal types: Galia and Piel de Sapo. The results were compared with those derived from a similar analysis carried out using agronomic traits. Our results indicated that RAPDs are useful in the establishment of the genetic relationships among melon genotypes as well as for germplasm management and improvement. The use of this data for the selection strategies of parental lines in melon hybrid production and for the evaluation of breeding programmes for these two varietal types is discussed.

Materials and methods

Plant material and sample preparation

Thirty-two breeding lines of melon (*Cucumis melo* L.), representatives of the Spanish cultivated germplasm, were analysed in this study: 14 belong to the Galia type (GOB, GOC, GOE, GOG, GOJ, GOK, GOX, G02, G06, G07, G09, Gll, G12 and G14) and 13 are included in the Piel de Sapo type (POA, POB, POC, POD, POF, POG, POH, P02, P03, P05, P10, Pll and P12). Five other lines from Charentais (FC4), Yellow (Y02), Rochet (R03), Japanese (FJ1) and American (FU2) varietal types were considered in this work. All the lines were provided by S & G Semillas (Novartis), and the plants were grown under identical greenhouse conditions.

Genomic DNA was extracted from young leaves following the procedure described by Dellaporta et al. (1983) and quantified by comparison with commercial standards after electrophoresis in agarose gels and ethidium bromide staining.

Polymerase chain reaction (PCR) reactions and primer selection

Amplification reactions were performed in a Perkin Elmer GeneAmp PCR System 2400. The conditions to create reproducible amplified DNA fingerprints were optimized through varying the different components of the reaction and the temperature profile. The primers used were obtained from Operon Technologies (Alameda, Calif.; kits A through N). The optimized reaction contained 10 ng DNA, 25 ng primer, 0.2 mM dNTPs, 1.5 U AmpliTaq DNA Polymerase Stoffel Fragment (Perkin Elmer), 10 mM TRIS-HCl, 2.5 mM $MgCl_2$ and 50 mM KCl, pH 8.3, in a 30-µl final volume. After 5 min of heating at 94°C, amplifications were performed under the following regime: 6 cycles of 15 s at 94°C, 15 s at 33°C, and 75 s at 72°C; followed by 35 cycles of 15 s at 94°C, 15 s at 37°C, and 75 s at 72° C, a final extension reaction of 7 min at 72 °C. PCR products were separated by electrophoresis in 1.5% agarose gels and stained with ethidium bromide.

Under these conditions, 280 primers were screened using 6 lines (GOK, G11, P11, POH, FJ1 and FC4) representative of the different types present in this analysis. Those primers showing no amplification, no consistent banding pattern or no polymorphism were rejected for subsequent analysis. After this first screening the primers selected were tried against the rest of the lines in two reaction sets, and the polymorphic ones showing a consistent pattern in all the lines were finally utilised in the statistical analysis. Only the strong reproducible bands produced by these primers were scored.

Analytical procedures

Each polymorphic band was scored as either present (1) or absent (0) for all genotypes, resulting in a binary data matrix. This matrix was used to calculate the Jaccard similarity coefficients (Jaccard 1908) and to generate the corresponding matrix showing genetic distance for each pair of lines. The unweighted pair-group method using arithmetic average (UPGMA) cluster analysis and the resulting dendrograms were performed on the genetic distance matrix using the computer programme NTSYS-pc version 1.80 (Rohlf 1997). On the other hand, presence/absence binary matrix was also employed

to perform a factor analysis with the STATGRAPHICS (1995) computer package. Factors representing the original variables were extracted by principal component analysis (PCA). Three-dimensional plots showing the relationships among the lines were generated from this analysis.

In order to compare the concordance between RAPD and agronomic data when assessing genetic relationships among the lines, the following agronomic traits were used: powdery mildew resistance, melon necrotic spot virus resistance, fusarium race-l resistance, fusarium race-2 resistance, sex determination, fruit size, rate length/diameter of the fruit, fruit endings, fruit peduncle, fruit net, fruit skin colour, turning fruit skin, fruit sutures, smoothness of the fruit skin, fruit dots, fruit spots, fruit flesh colour, seed section shape, plant vigour under cold conditions, earliness to ripening and climatery. A new binary matrix of presence/absence was then generated considering 2 markers for each trait. Lines showing extreme phenotypes were scored as (O/l) or (l/O), whereas intermediate phenotypes were scored as (l/l). This new matrix was subjected to the same analysis as the previous one based on RAPDs. The level of correlation between the elements of the RAPD and agronomic distance matrices was determined using the Mantel test (Mantel 1967), which assumes that the two matrices were obtained independently. Calculations were made using the NTSYS-pc version 1.80 (Rohlf 1997).

Results

To select primers showing both higher levels of polymorphism and reproducible patterns, we made a preliminary analysis using a total of 280 primers and 6 representative lines (see Materials and methods). Using this approach, we selected 123 primers as candidates to detect polymorphism among the total 32 lines. After those 123 primers were tested on the 32 lines, only 43 were found to produce intensely stained and reproducible polymorphic bands after two PCR reactions. These primers were selected and yielded 234 bands (an average of 5.4 bands each), 115 (49%) of which were polymorphic among the lines analysed. The amplified fragments ranged in size from approximately 200-2000 bp. An example of the amplification products obtained with 20 of these lines using primer OPB-17 is shown in Fig 1.

Results from our test for presence or absence of these 115 polymorphic fragments were used to generate the genetic distance matrix shown in Table 1 (above diagonal). ranged from 0.107 for the most related lines of the Piel de Sapo type (PO5 and P02) to 0.727 for the more distant ones (PO5 and FC4). Considering the two varietal types separately, Galia lines were relatively more divergent from each other (average distance of 0.406; range: 0.159–0.526) than Piel de Sapo ones (average distance of 0.328; range: 0.107-0.536). UPGMA clustering analysis was carried out in order to represent graphically the genetic distances among melon lines. The resulting dendrogram showed two main groups (Fig. 2). The first group included the Piel de Sapo lines together with the Yellow and Rochet representatives. The second one comprised Galia lines together with the Japanese, American and Charentais representatives.

To display the relationships among the melon lines in term of their positions relative to three coordinate axes, we also performed principal component analysis (PCA). The first three principal factors derived from this analysis explained 59% of the observed variation and allowed for a clear distinction of the varietal types (Fig. 3A). In agreement with the results of the dendrogram, Galia and Piel de Sapo representatives were clustered in two major groups. Rochet and Yellow types were located in an intermediate position between Galia and Piel de Sapo types, whereas Charentais and American were closer to Galia lines than to Piel de Sapo ones. The Japanese genotype was integrated in the Galia cluster. The three-dimensional plot also showed that the lines from Piel de Sapo were less disperse than those from Galia, indicating a lower genetic variability among Piel de Sapo than among Galia lines.

We also established the similarities among melon breeding lines on the basis of their agronomic features. A distance matrix of the 32 lines was generated for data corresponding to 24 agronomic traits covering features of the plant and fruit (Table 1, below diagonal), and this was compared with the genetic distance matrix obtained from RAPD data. A regression analysis performed by means of a Mantel test (Fig. 4) indicated a high correlation between molecular and agronomic markers in their ability to detect genetic relationships

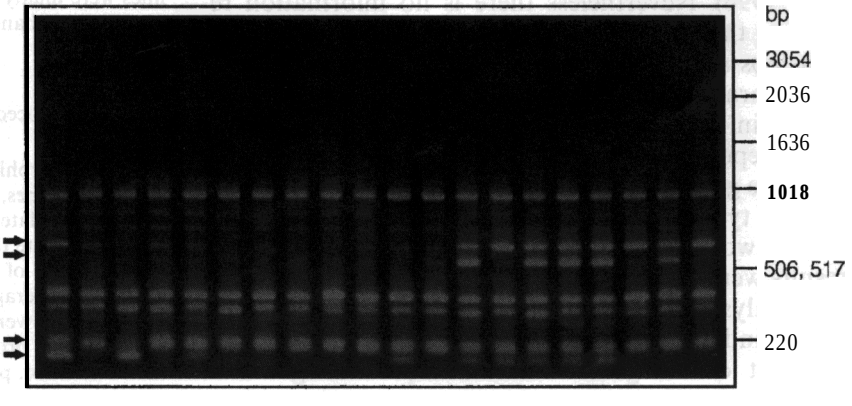

Fig. 1 RAPD patterns of 20 lines of C. *melo* analysed with primer OPB-17. Scored polymorphic RAPD bands are marked (arrows). Size markers are given in base pairs (bp)

Table 1 Pairwise distance values between melon genotypes included in several varietal types. Calculations were based on RAPD (above diagonal) and agronomic data (below diagonal)

RAPD distances

Varietal types		Galia														Piel de Sapo													Charentais	Japanese	American	Rochet	Yellow
		GO2	GO6	GO7	GO9	G11	G12	G14	GOB	GOC	GOE	GOG	GOJ	GOK	GOX	PO2	PO3	PO5	P10	P11	P12	POA	POB	POC	POD	PDF	POG	POH	FC4	FJ1	FU2	R03	Y02
Galia	GO2		0.342	0,352	0,347	0.278	0,466	0,450	0,265	0,338	0,333	0,310	0,447	0,419	0,269	0,655	0,580	0,646	0,634	0.642	0,613	0,500	0,646	0,557	0,575	0,521	0,584	0,612	0,522	0,494	0,489	0,532	0,570
	GO6	0,200		0,209	0,159	0.211	0,487	0,329	0,506	0,408	0,468	0,243	0,429	0,462	0,434	0,585	0,578	0,593	0,598	0,588	0,627	0,595	0,538	0,538	0,519	0,595	0,582	0,576	0,522	0,459	0,474	0,513	0,550
	G07	0,233	0,107		0,268	0,315	0,479	0,423	0,500	0.419	0,459	0,224	0,419	0,474	0,403	0,600	0,557	0,625	0,613	0,603	0,659	0.573	0,590	0,570	0,532	0,610	0,597	0,590	0,565	0,432	0,516	0,545	0,564
	GO9	0,324	0,161	0,250		0,289	0,506	0,375	0,506	0,410	0,488	0,297	0,450	0,481	0,475	0,583	0,560	0,590	0,595	0,585	0,607	0,593	0,556	0,537	0,519	0,575	0,563	0,558	0,505	0,442	0,442	0,494	0,583
	G11	0,207	0,207	0,241	0,226		0,506	0,415	0,410	0,524	0,468	0,297	0,430	0,443	0,373	0,632	0,640	0,655	0,628	0,651	0,624	0.575	0,607	0,605	0,588	0,593	0,598	0.637	0,438	0,424	0,409	0,583	0,531
	G12	0,500	0,364	0,344	0,219	0,424		0,447	0,444	0,486	0,507	0,459	0,526	0,437	0,514	0,628	0,547	0,618	0,641	0,595	0,581	0,563	0,600	0,579	0,615	0,543	0,608	0,617	0,589	0,494	0,538	0,471	0.493
	G14	0,333	0,281	0,364	0,188	0,344	0,382		0,506	0,347	0.449	0,342	0,390	0,423	0,475	0,434	0,449	0,461	0,447	0,432	0,461	0,519	0,440	0,400	0,442	0,459	0,446	0,450	0,521	0,460	0,490	0,413	0,434
	GOB	0,226	0,382	0,313	0,432	0,394	0,514	0,444		0,384	0,446	0,481	0,506	0,461	0,343	0,686	0,580	0,694	0,617	0,642	0,630	0,521	0,630	0,575	0,610	0,541	0,584	0,612	0,522	0,439	0,489	0,551	0,605
	GOC	0,344	0,344	0,323	0,353	0,300	0,486	0,364	0,258		0,258	0,400	0,506	0,397	0,411	0,570	0,446	0,558	0,564	0,533	0,595	0,500	0,558	0,459	0,459	0,541	0,547	0,487	0,538	0,512	0,505	0,431	0,588
	GOE	0,375	0,375	0,300	0,382	0,333	0,471	0,394	0,233	0,038		0,375	0,487	0,348	0,452	0,590	0,526	0,579	0,603	0,610	0,633	0,542	0,615	0,577	0,577	0,562	0,641	0,580	0,571	0,563	0,537	0,514	0,533
	GOG	0,394	0,290	0,207	0,303	0,300	0,394	0,412	0,258	0,143	0,111		0,378	0,370	0,405	0,563	0,556	0,588	0,593	0,600	0,622	0,533	0,570	0,550	0,568	0,514	0,558	0,571	0,533	0,471	0,468	0,544	0,487
	GOJ	0,553	0,429	0,500	0,294	0,486	0,382	0,400	0,486	0,412	0.441	0,364		0,461	0,389	0,622	0,580	0,646	0,600	0,625	0,663	0,615	0,663	0,643	0,610	0,633	0,687	0,628	0,585	0,512	0,536	0,622	0,588
	GOK	0,455	0,406	0,485	0,412	0,419	0,583	0,375	0,424	0,214	0,250	0,333	0,375		0,403	0,582	0,575	0,608	0,646	0,584	0,625	0,573	0,590	0,605	0,639	0,573	0,615	0,607	0,565	0,557	0,500	0,545	0,526
	GOX	0,343	0,242	0,324	0,206	0,400	0,343	0,314	0,447	0,500	0,526	0,459	0,265	0,514		0,679	0,588	0,671	0,642	0,615	0,620	0,507	0,638	0,582	0,600	0,587	0,592	0,635	0,544	0,500	0,526	0,558	0,558
Piel de Sapo	PO2	0,667	0,667	0,658	0,625	0,780	0,514	0,675	0,675	0,756	0,750	0,756	0,707	0,744	0,600		0,281	0,107	0,217	0,306	0.369	0,529	0.262	0,373	0,297	0,415	0,375	0,313	0,720	0,602	0,644	0,412	0,435
	PO3	0,707	0,707	0,700	0,600	0,756	0,486	0,650	0,650	0,667	0,658	0,667	0,615	0,649	0,643	0,179		0,258	0,242	0,246	0,338	0,385	0,313	0,292	0,318	0,359	0,441	0,284	0,673	0,580	0,650	0,451	0,514
	PO5	0,692	0,692	0,684	0,650	0,805	0,543	0,700	0,700	0,780	0,775	0,780	0,732	0,769	0,625	0,040	0,214		0,220	0,311	0,349	0,536	0,323	0,354	0,274	0,422	0,406	0,318	0,727	0,625	0,663	0,369	0,441
	P10	0,538	0,538	0,564	0,500	0,659	0,500	0,550	0,585	0,634	0,659	0,667	0,619	0,615	0,475	0,167	0,250	0,200		0,295	0,306	0,478	0,306	0,286	0,313	0,381	0,391	0,250	0,717	0,598	0,653	0,471	0,449
	PI1	0,650	0,615	0,605	0,575	0,732	0,457	0,625	0,659	0,738	0,732	0,707	0,625	0,692	0,513	0,143	0,233	0,179	0,219		0,224	0,413	0,311	0,317	0,344	0,333	0,397	0,308	0,670	0,636	0,660	0,433	0,542
	P12	0,683	0,650	0,641	0,538	0,700	0,412	0,590	0,690	0,707	0,700	0,675	0,590	0,658	0,550	0,207	0,172	0,241	0,273	0,071		0,371	0,349	0,274	0,379	0,288	0,328	0,318	0,674	0,625	0,650	0,486	0,486
	POA	0,585	0,585	0,610	0,475	0,634	0,513	0,487	0,487	0,459	0,486	0,500	0,405	0,472	0,488	0,444	0,324	0,472	0,306	0,432	0.389		0,371	0,400	0,448	0,250	0,350	0,412	0,691	0,612	0,667	0,569	0.549
	POB	0,738	0,738	0,732	0,634	0,786	0,568	0,683	0,615	0,632	0,622	0,632	0,579	0,611	0,674	0,300	0,207	0,276	0,400	0,394	0,344	0,273		0,274	0,328	0,317	0,300	0,292	0,727	0,593	0,650	0,441	0.486
	POC	0,744	0,744	0,738	0,643	0,791	0,615	0,690	0,625	0,641	0,632	0,641	0,590	0,622	0,682	0,323	0,233	0,355	0,371	0,412	0,364	0,294	0.172		0,226	0,349	0,279	0,299	0,670	0,541	0,606	0,397	0,443
	POD	0,762	0,762	0,756	0,659	0,810	0,595	0,707	0,641	0,658	0,649	0,658	0,605	0,639	0,698	0,276	0,179	0,250	0,382	0,375	0,323	0,303	0,038	0,143		0,400	0,279	0,324	0,684	0,575	0,606	0,348	0,466
	POF	0,825	0,625	0,615	0,513	0,675	0,429	0,564	0,466	0,500	0,486	0,500	0,444	0,556	0,561	0,394	0,313	0,424	0,432	0,429	0,382	0,212	0,200	0,281	0,233		0,263	0,286	0,691	0,578	0,653	0,485	0,485
	POG	0.585	0,585	0,610	0,475	0,634	0,474	0,525	0,487	0,459	0,486	0,500	0,405	0,514	0,524	0,400	0,273	0,429	0,306	0,432	0,389	0,063	0,219	0,242	0,250	0,156		0,373	0,722	0,566	0,657	0,424	0,493
	POH	0,707	0,707	0,700	0,600	0,756	0,486	0,650	0,579	0,595	0,583	0,595	0,541	0,571	0,643	0,300	0,207	0,333	0,353	0,344	0,290	0,324	0,207	0,233	0,179	0,138	0,273		0,643	0,545	0,594	0,513	0,493
Charentais	FC4	0,459	0.500	0,526	0,537	0.514	0,643	0,550	0,550	0,634	0,659	0,600	0,550	0,615	0,395	0,750	0,756	0,773	0,636	0,674	0,705	0,674	0,783	0,761	0,804	0,739	0,702	0,756		0,446	0,275	0,638	0,653
Japanese	FJ1	0,323	0,375	0,406	0.429	0,438	0,471	0,394	0,486	0,500	0,529	0,543	0,528	0,559	0,353	0,684	0,725	0,711	0,590	0,632	0,667	0,634	0,786	0,818	0,810	0,675	0,667	0,725	0,472		0,400	0,602	0,518
American	FU2	0,382	0,382	0,457	0,474	0,441	0,625	0,444	0,526	0,500	0,528	0,579	0,564	0,424	0,405	0,738	0,773	0,762	0,619	0,690	0,721	0,689	0,800	0,778	0,822	0,756	0,717	0,773	0,343	0,344		0,602	0,558
Rochet	R03	0,585	0,474	0,500	0,351	0,526	0,389	0,447	0,561	0,500	0,526	0,459	0,361	0,429	0,410	0,486	0,371	0,514	0,351	0,369	0,343	0,324	0,459	0,432	0,444	0,447	0,368	0,417	0,581	0,600	0,595		0,521
Yellow	Y02	0,650	0,579	0,568	0,538	0,700	0,457	0,625	0,659	0,738	0,732	0,675	0,514	0,692	0,389	0,323	0,394	0,355	0,371	0,200	0,258	0,513	0,486	0,500	0,471	0,514	0,513	0,486	0,575	0,595	0,625	0,343	

Agronomic distances

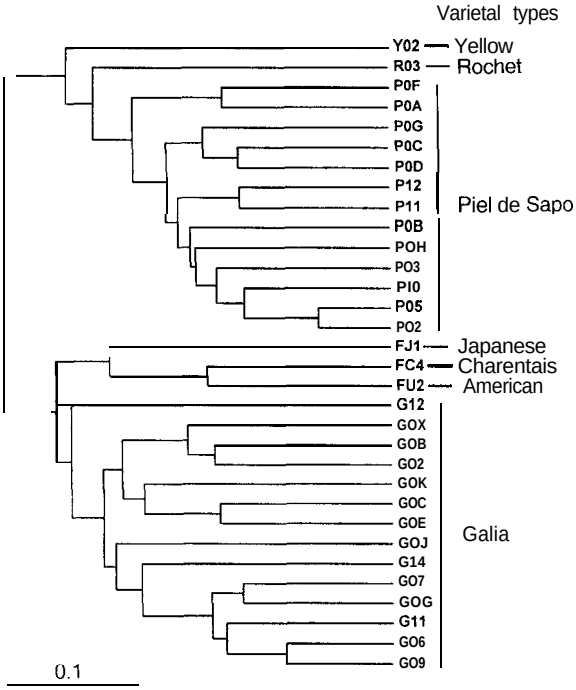

Fig. 2 Associations among *C.melo* lines revealed by U PGMA cluster analysis on the basis of RAPD genetic distance values

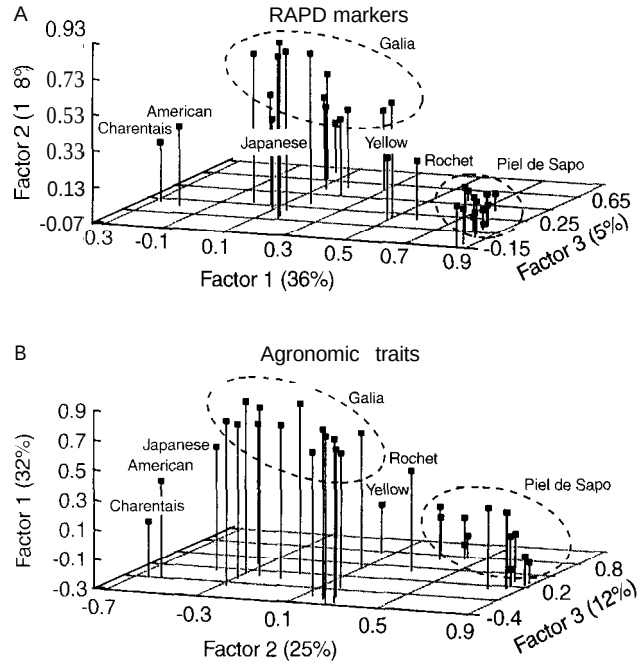

Fig. 3A,B Ordenation of the 32 lines of melon after principal component analysis (PCA) using RAPD markers (A), and agronomic traits **(B)**

between melon lines ($r = 0.79$; $t = 17.03$; $P < 0.001$). Moreover, the relationships among the lines revealed by UPGMA (data not shown) and PCA were very similar to those obtained from RAPDs (Fig. 3A and B). Nevertheless, it should be pointed out that the dispersion of Piel de Sapo lines in the three-dimensional plot is higher than that detected with RAPDs, suggesting a lower genetic variability among Piel de Sapo lines than was expected, given their agronomic features.

Lines from Galia and Piel de Sapo were analysed separately in order to assess the ability of both RAPDs and agronomic traits to reveal the evolution of the known breeding programmes from which they derived. With this purpose, two additional data matrices were generated for each varietal type, one from agronomic data and the other from RAPDs. PCA from agronomic data clearly distinguished two clusters in both Galia and Piel de Sapo (Fig. 5A and B) which resemble their agricultural characteristics. This is not surprising since the lines of both varietal types were derived from a breeding programme trying to develop two separated groups of parental lines to be used in hybrid production. In the group coinciding with the cluster formed by the lines G02, G06, G07, G09, G11, G14 and GOX in Galia and lines P02, P03, P05, P10, P11 and P12 in Piel de Sapo (hereafter referred to as group A), important agronomic characteristics such as plant vigour or climatery degree of the fruits are being maintained by selection. In the other group, corresponding to the cluster formed by the lines GOB, GOC, GOE, GOG, GOJ, GOK and G12 in Galia and lines POA, POB, POC, POD, POF, POG and POH in Piel de Sapo (hereafter referred to as group B), lines were developed and selected on the basis of their resistance to different pathogens, such as powdery mildew or fusarium wilt.

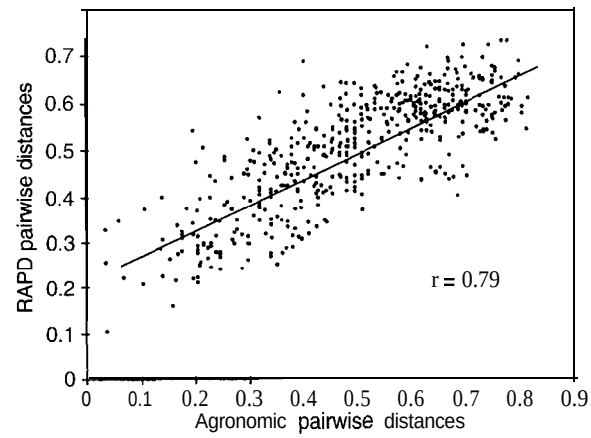

Fig. 4 Regression analysis to compare the genetic pairwise distances obtained from RAPD markers and agronomic traits

As shown in Fig. 5C and D, these two groups were also detected from the PCA analysis based on RAPD markers. In Galia type, these groups were clearly separated from each other, indicating an apparent genetic divergence between the lines included in each one

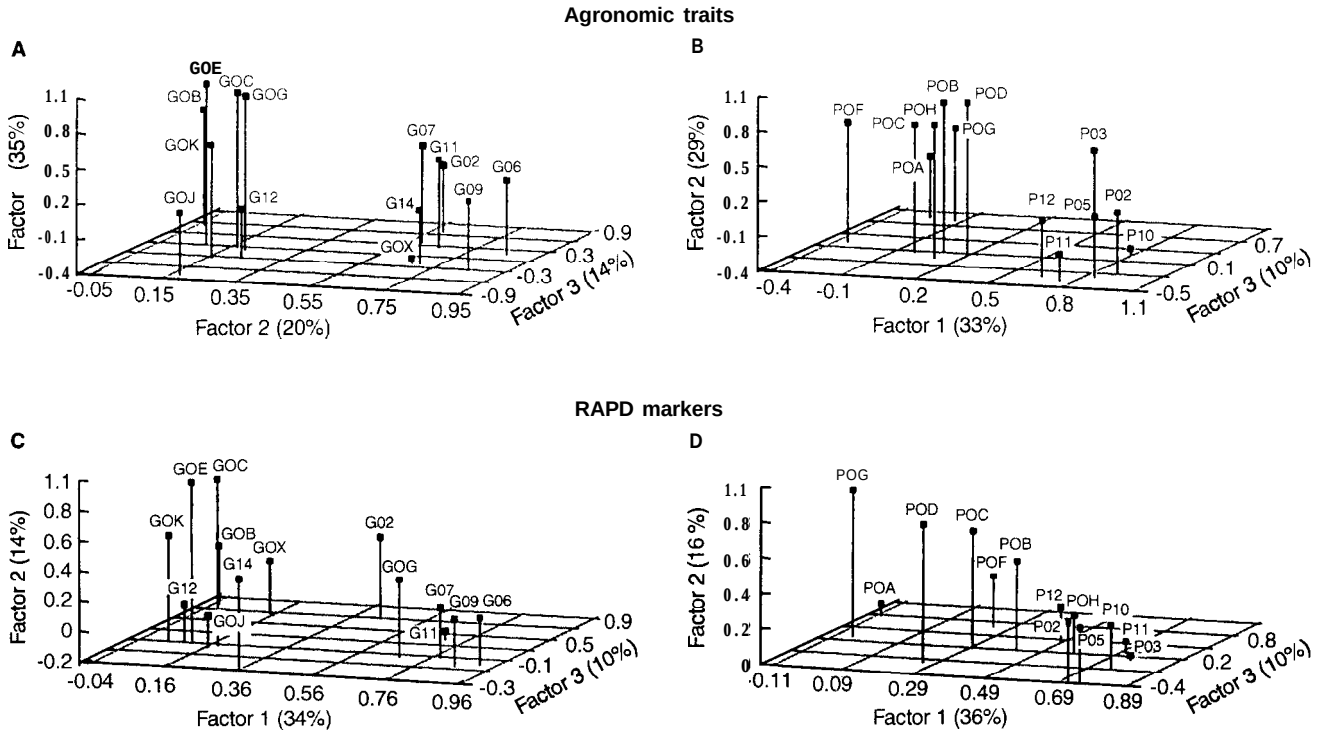

Fig. 5A–D Plots derived from PCA in Galia **(A** and C) and Piel de Sapo (B and **D)** lines. Groupings of the lines were based on agronomic traits **(A** and B), and RAPD markers (C and D)

(Fig. 5C), as was expected from the breeding programme. Only the lines GOG, GOX and G14 were clustered in a group which does not correspond to their main agronomic features. In Piel de Sapo type, however, these two groups seemed not to be so evident as in Galia (Fig. 5D). In addition, the line POH, included in the group A on the basis of its agronomic features, was clustered in the group B on the basis of the RAPD molecular data.

Discussion

A final selection for primers producing a higher level of polymorphism and more reproducible banding patterns, was facilitated by first performing a preliminary screening using a reduced number of lines and a large number of primers. Given the results, our study indicates the usefulness of such screening, not only to save time and expense but essentially to reject primers that are not informative for the analysis. Such preliminary screening has also given successful results in others species (Demeke et al. 1992; Schnell et al. 1995; Abo-elwafa et al. 1995). Once identified, the selected primers could also serve to evaluate a larger number of melon accessions in future analysis. We have used the information generated by these primers for identifying markers tightly linked to agronomic importance traits in F_2 segregating populations derived from crosses between some of the parental lines included in this analysis. In addition, the polymorphisms found among the parental lines are now being used in hybrid purity tests to detect the occurrence of non-desired pollinations in the F_1 hybrid production.

Despite the low level of genetic polymorphism existing in the C. *melo* genome (Shattuck-Eidens et al. 1990), RAPD markers have proved to be informative enough to allow for the evaluation of representative germplasm accessions of cultivated melon. The amount of genetic variability detected by RAPDs among the lines analysed in this study (49% polymorphism) is higher than that detected by RFLPs (Neuhausen 1992) and isozyme markers (Perl-Treves et al. 1985). Using RAPDs, Baudracco-Arnas and Pitrat (1996) and Katzir et al (1996) detected only 18.3% and 38% of polymorphic markers, respectively, although they used a reduced number of accessions. In addition, the accessibility of the methodology and the fact that RAPDs are widely distributed throughout the genome of melon (Baudracco-Arnas and Pitrat 1996) clearly support the utility of these markers for melon genome analysis and germplasm evaluation.

In both the dendrogram produced from UPGMA clustering (Fig. 2) and the three-dimensional plot derived from PCA (Fig. 3A) a number of melon accessions were separated into different groups on the basis of 115 polymorphic RAPD markers. This grouping agrees with the botanical classification of varieties

proposed by Naudin (Whitaker and Davis 1962). The cluster on the left includes the Charentais (FC4) and American (FU2) representatives, both with orange flesh and climacteric fruits included in the variety '*Cantalupensis*'. The next cluster is formed by Galia lines and the Japanese representative (FJ1). Galia type is a green flesh melon included in the variety *'Reticulatus'*, while the Japanese has been classified as intermediate between *'Cantalupensis'* and *'Reticulatus'*. The cluster located most to the right groups lines of Piel de Sapo, a white flesh, non-climateric type of melon included in the variety '*Inodorus*'. The position of the Rochet (R03) and the Yellow (Y02) representatives agrees with their inclusion in *'Saccharinun'*, a variety with characteristics intermediate between those of '*Reticulatus*' and *'Inodorus'*.

As well as distinguishing among different varietal types, our analysis also detected the relationships of lines within each varietal type. In this sense, RAPD data are congruent with the pedigree information we have available from melon breeding programmes. In Galia, the programme started with material from two different sources. Lines G06, GO7 emerged by inbreeding of the original Galia type (Galia F_1). Different backcrosses of these lines gave rise to the lines grouped in the cluster with agronomic traits (group A) but without resistance characteristics (GO9 and G11). The other cluster derived from GOB and GOK after dominant resistance genes (particularly against powdery mildew and fusarium) were introduced to the Galia type. Additionally, the introgression of other agronomic features from Rochet and Japanese lines resulted in the lines GOJ, GOE and GOC that cluster in group B. On the other hand, Piel de Sapo lines, which are derived from Spanish traditional cultivars, were initially crossed with an Ogen type for the introgression of some resistance and quality characters. From the first line that emerged, POA, successive backcrosses originated two sets of lines: (1) POG, POB and POD having in common certain resistance genes, and (2) P11, P10, P05, PO2 and PO3 that share the majority of agronomic traits. According to this pedigree data, lines sharing any parental line tend to be clustered together on the basis of RAPD markers (compare Figs. 2 and 5).

In the present study we have found a high correspondence between RAPDs and agronomic traits when assessing the genetic relationships among melon lines. The correlation between the distances estimated from both kinds of data was 0.79, a value that is higher than that detected in other species such as cotton when genetic distances from RAPDs and morphological characters were compared (Tatineni et al. 1996). Furthermore, in agreement with results obtained in other species (Hoey et al. 1996), the clusters of lines produced by RAPD data are very similar to those generated by agronomic traits (see Fig. 3). RAPDs detected a lower genetic divergence among Piel de Sapo lines than did the agronomic characteristics (compare Fig. 3A and B). The results from RAPDs seem to be more reliable since these markers cover a larger proportion of the genome, including coding and noncoding regions, and have not been subjected to artificial selection. Therefore, these results may indicate that the objective of the breeding programmes – trying to obtain two divergent groups of parental lines – appear to have not yet been achieved in Piel de Sapo. In fact, when Piel de Sapo lines were analysed separately on the basis of RAPD markers, these two groups were not so clearly defined as in Galia (Fig. 5).

There are numerous studies that support the existence of a positive correlation between the genetic divergence of parental lines and hybrid performance in a number of species (Lee et al. 1989; Smith et al. 1990; Zhang et al. 1995). After clustering analysis in Galia and Piel de Sapo, RAPD markers were able to separate two groups of parental lines (Fig. 5). Only the lines GOG, GOX and G14 in Galia and the line POH in Piel de Sapo were grouped in a different cluster to that corresponding to the ordination based on agronomic characteristics. Although we have not made any measure of the performance or heterosis in the hybrids derived from the analysed lines, there are some data that should be mentioned. Crosses in Galia between distant lines such as G12 x GOB (RAPD distance, RD = 0.444; agronomic distance, AD = 0.514) and GO9 x GOJ (RD = 0.450; AD = 0.294) have produced excellent hybrids which are in fact commercial products at the moment, as is the case of POF x PO5 (RD = 0.422; AD = 0.424) in Piel de Sapo. On the other hand, crosses between lines that, although apparently unrelated on the basis of their agronomic characterization, are less divergent at the RAPD level, have produced hybrids which have been rejected because of their low performance. This is the case of the hybrid derived from G14 x GOG (RD = 0.342; AD = 0.412) in Galia, or POB x PO2 (RD = 0.262; AD = 0.300) in Piel de Sapo. The lines COG and G14 involved in these crosses were differently clustered by RAPDs and agronomic features, which indicates that RAPDs provide a better knowledge of the genetic relationships existing among the lines than agronomic characters, the former being of interest in the selection of appropiate parental lines for commercial hybrid production.

Taken as a whole, these results clearly prove the applicability of RAPD markers not only to establish the genetic relationships of melon germplasm, confirming pedigree data even among closely related lines, but also in melon germplasm management and breeding programme purposes. Compared with morphological characters, RAPDs thus seem to be more appropriate markers to determine the genetic relations existing among parental lines in melon and therefore to predict the performance and heterosis of hybrids derived from these lines. This will be of utility in current breeding programmes of this species since field screening of diallelic crosses are both time- and labour-consuming.

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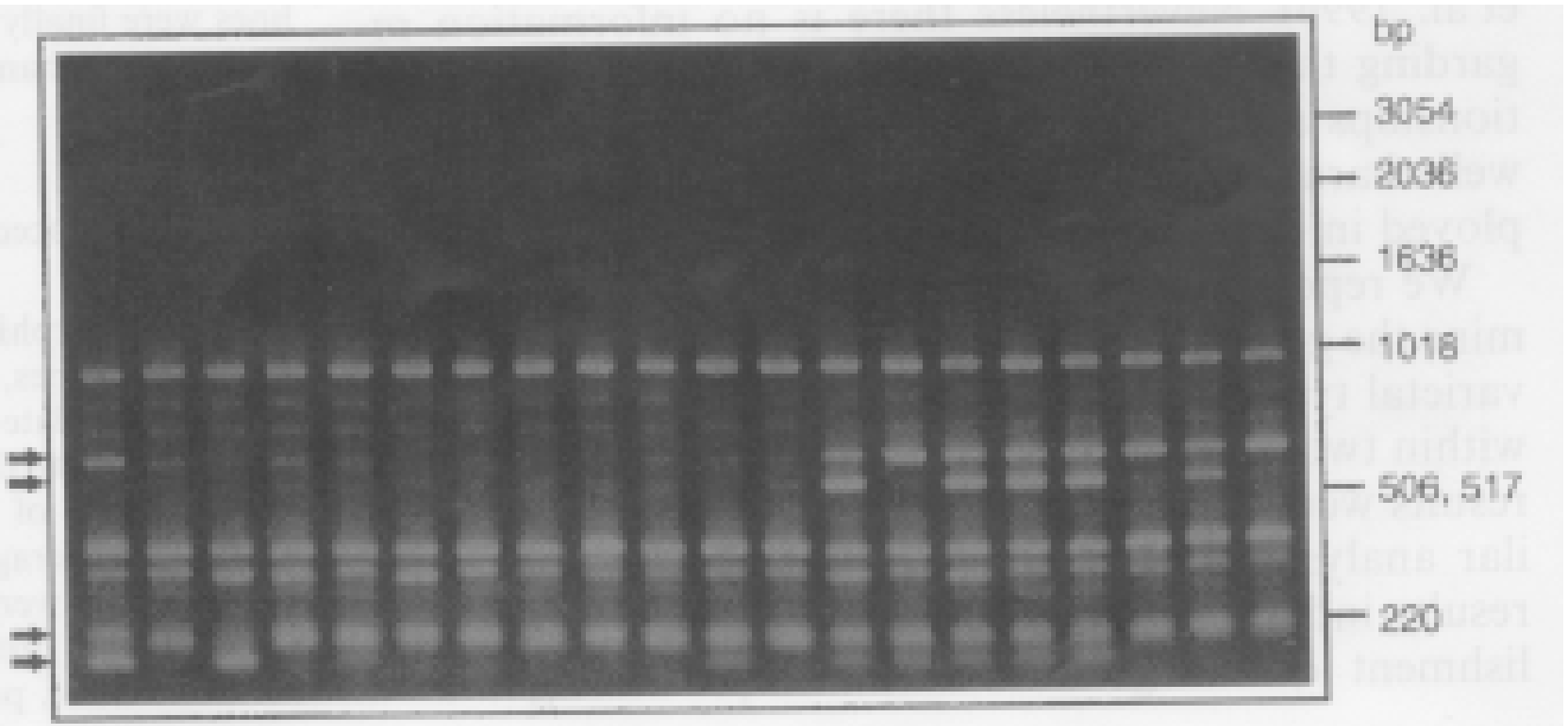
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