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Numerical analyses of RAPD data highlight the origin of cultivated tagasaste (*Chamaecytisus proliferus* ssp. *palmensis*) in the Canary Islands

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Abstract. Random Amplified Polymorphic DNA (RAPD) was used to generate molecular markers to trace the origin of the fodder legume tagasaste (*Chamaecytisus proliferus* (L. fil.) Link ssp. *palmensis* (H. Christ) Kunkel) in the Canary Islands. Results from multivariate analyses of data through "Two Way Indicator Species Analysis" (TWINSPAN) and "Detrended Correspondence Analysis" (DECORANA) showed that genotypes collected on the island of La Palma exhibited a wider range of variation than those from the other islands. This supports the existing hypothesis that tagasaste originated on La Palma and emphasizes the importance of conserving and evaluating germ plasm from this island.

Key words: *Chamaecytisus* – Tagasaste – Fodder legumes – Multivariate analysis – RAPD – Plant genetic resources – Biodiversity

Introduction

The legume shrub of the tribe Genisteae known as tagasaste (*Chamaecytisus proliferus* (L. fil.) ssp. *palmensis* (H. Christ) Kunkel) is one of the few endemic species of the Canary Islands that is cultivated (Francisco-Ortega 1992). It has gained economic importance not only in the Canary Islands but also in New Zealand and Australia where it was introduced late in the last century and is now used to feed livestock during the summer when other fodder is scarce (Francisco-Ortega et al. 1991, 1992c).

Early reports on the history of tagasaste as a cultivated species indicate that this fodder crop originated from the island of La Palma and was introduced to the island of Tenerife in 1856 by Victor Pérez (Pérez-Ventoso 1892; Morris 1893). Pérez provides the first account of the traditional utilization of tagasaste in La Palma (Pérez 1862a, b) and was also responsible for its introduction to Australia (Francisco-Ortega et al. 1991). Tagasaste forms a taxonomic complex within which the other forms are not cultivated and are locally known as 'escobon' (Acebes-Ginovés et al. 1991).

Following the development of the polymerase chain reaction (PCR) a new approach for analysing patterns of genetic variation has arisen through the generation of random amplified polymorphic DNA markers (RAPDs) (Williams et al. 1990; Newbury and Ford-Lloyd 1993). This technique offers a further way to 'fingerprint' genotypes and to obtain molecular markers that can be useful in studies of crop evolution and genetic resources.

In this paper we describe the use of RAPD to detect genetic variation in the germ plasm of wild and cultivated tagasaste from the Canary Islands. We use these results to confirm the origin and spread of tagasaste in the Canary Islands and to establish conservation priorities for the plant genetic resources of this fodder species. We also demonstrate that some multivariate methods traditionally used by ecologists to study floristic data can be useful for the analysis and interpretation of RAPD data.

Materials and methods

Plant material

The 20 population samples of tagasaste are described in Table 1. These germ plasm accessions are currently held in the Centro de Conservación de Recursos Fitogenéticos, Madrid, and further information concerning the ecology of the collection localities

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Table 1. The 20 accessions of tagasaste used in this study. The accession numbers listed are those of the Birmingham University collection. Accessions from wild populations are indicated with an asterisk

Accession no.	Collection site	Island	Accession no.	Collection site	Island
3	Malpaises	La Palma	20	Casas de La Mata	La Palma
5	Tomascoral	La Palma	*23	Los Tilos	La Palma
6	Casa Forestal de El Paso	La Palma	*26	Canal de Marcos	La Palma
7	La Cumbrecita	La Palma	27	Las Cabezadas	La Palma
8	Valencia	La Palma	28	Roque Faro	La Palma
9	Hoya de la Sima	La Palma	37	Utiaca	Gran Canaria
10	El Tunel de El Paso	La Palma	97	La Montañeta	Gran Canaria
11	Montaña Paloma	La Palma	121	Las Portelas	Tenerife
12	Barranco de Madera	La Palma	133	Aguamansa	Tenerife
15	Casas de Taburiente	La Palma	175	La Peña	El Hierro

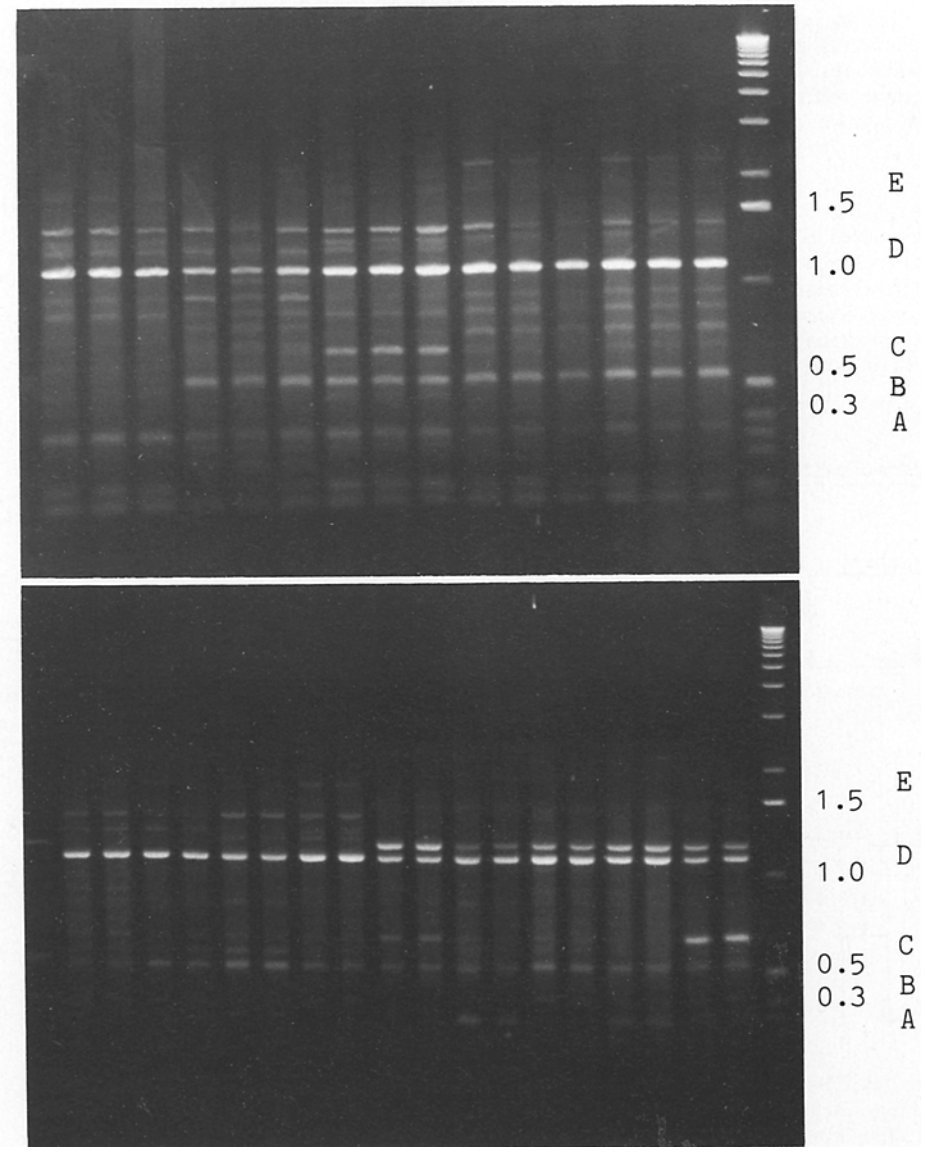

Fig. 1. Single primer PCR on genomic DNA from 2 accessions of tagasaste. Zones of activity (*extreme right-hand column*) and molecular weight marker are shown

can be found in Francisco-Ortega et al. (1990, 1992a). Cultured tagasaste embryos were grown to seedlings for 1 month (Reghunath et al. 1993) and DNA extracted from 0.15-g (fresh weight) samples following the protocol of Gawel and Jarret (1991). The amount of genomic DNA was measured with a TKO 100 mini-fluorometer using Hoechst dye according to the manufacturer's instructions. At least five individual plants per accession were studied.

Polymerase chain reaction

The PCR was carried out in a 50-µl reaction volume containing Boehringer reaction buffer, 0.1 m*M* of each deoxyribonucleotide triphosphate, 0.2 n*M* primer, 1 unit Taq polymerase (Boehringer), 2.5 m*M* $MgCl_2$ and 15 ng template tagasaste DNA. The reaction mix was covered with mineral oil. The single ten-base primer employed had the following sequence: CGGGCGATAT. A Hybaid Thermal Reactor HBTR1 was used for amplification, and it was programmed with an initial cycle of 7 min at 94 °C, 1 min at 36 °C and 4 min at 72 °C. The successive 35 cycles were identical except that the 94 °C treatment was for only 1 min. The separation of PCR products was conducted by electrophoresis for 4 h at 80 V in 1.0% agarose gels using TBE buffer, and the DNA fragments were visualized using ethidium bromide staining (Maniatis et al. 1982). Gels were photographed using UV light Polaroid film 667. Each amplification was executed 3 times, and only those bands which were subsequently consistent in these three amplifications were considered for multivariate analysis.

Data analyses

For the multivariate analyses DNA fragments detected after electrophoretic separation as bands were scored as present (+) or absent (−). A data matrix based on DNA fragments detected per genotype was constructed. A hierarchical classification was obtained after using 'Two Way Indicator Species Analysis' (TWINSPAN) (Hill 1979a). 'Detrended correspondence analysis' (DECORANA) (Hill 1979b) was used for ordination. These multivariate analyses were carried out using the Cep-pc package (Mohler 1987).

Results

An initial survey had previously been carried out using eight primers and five accessions. These preliminary results revealed that the primer subsequently used in this research yielded the highest levels of information and diversity. Using this single primer we detected a total of 30 DNA amplification products. These were arbitrarily divided into the five zones (A–E) of different molecular weight (Fig. 1). A total of 79 different genotypes were identified within the 20 accessions. Twelve PCR reactions did not produce readable tracks.

The hierarchical classification generated by TWINSPAN (Fig. 2) yielded 29 End Groups (final clusters that were significant). Genotypes found in these End Groups are shown in the re-ordered Genotype-by-DNA Fragment Data Matrix that was obtained after this classification (Table 2). Results from this analysis were in agreement with those from a revious Cluster Analysis as accessions which were not collected in La Palma only clustered in one of the major groups formed at the highest division (Fig. 2); they were also placed together on the re-ordered data matrix (Table 2). No genotype from the other islands was found in End Groups 17–29, whereas only End Groups 1, 5 and 6 did not have genotypes from La Palma. Genotypes which did not come from La Palma predominantly possessed DNA frag-

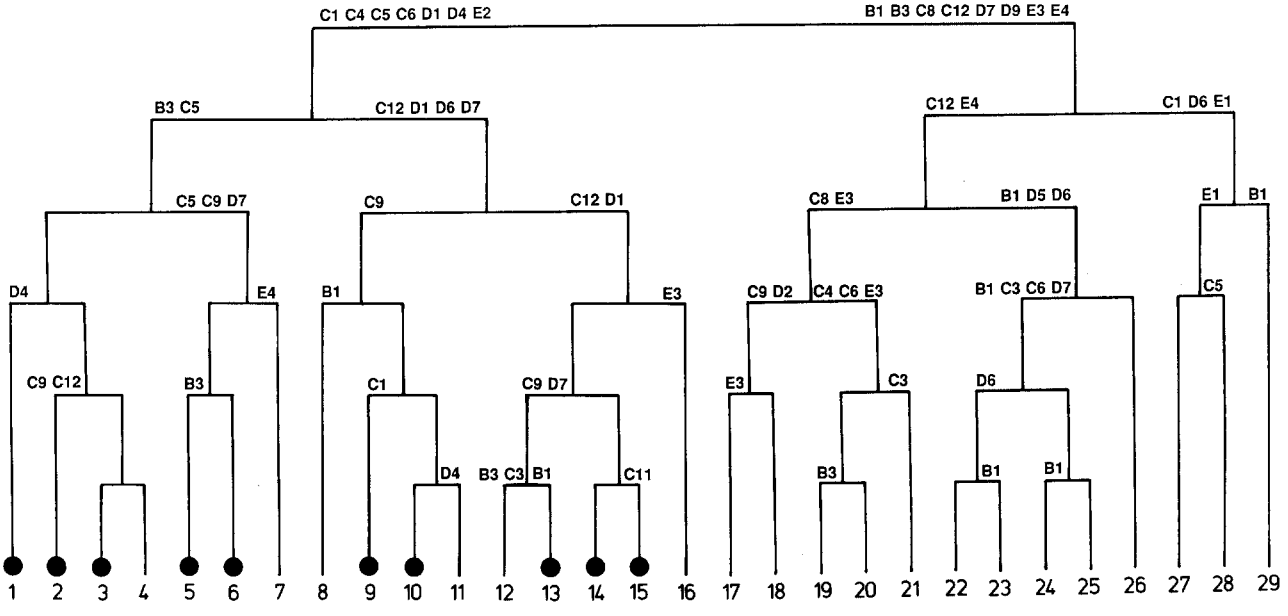

Fig. 2. The 29 End Groups obtained after TWINSPAN analysis of DNA fragments from 20 accessions of tagasaste. Diagnostic DNA fragments at each division are given, and they can also be found in Table 2. Genotypes found in each End Group are given in Table 2. *Closed circles* indicate End groups with some or all of the genotypes that were not collected on La Palma

Table 2. 'Bandmap' or Genotype-by-DNA Fragment Data Matrix interpreted from the TWINSPAN analysis of 20 accessions of wild and cultivated tagasaste. The accession numbers listed are those of the Birmingham University collection; these are indicated on the upper heading. Group no. = End Group number obtained after TWINSPAN classification (Fig. 2); these are given on the lower heading. DNA fragments are given on left column. Asterisks are used for those accessions which were not collected on La Palma

	*** * * ***** ** *** * ***
Accession no.	1 11111 1 1 1 1 1
	333 19221117773311 23922299 221111393111 2 2111 222 2222222222
	7776576120055533006986373317756660155553731113383302223300888955888777876677799
Group no.	1112223334455566778899911111111111111111111111111111111222222222222222222222222
	0000012222334444456667788889999012333344445666677888999
C5	111111111
D1	11 1 111111111 1111 1 1
C1	111 1 1 1111111111 11 1111111111 1111 1 1 111111
D4	111 1 1 1 111 111 1 11 1 1
E2	1 1 1 1 1 1
C4	1 1 111111111111111 11111111111 11 1 111111 11 1111 111 111 1 11111
C6	111111 111 1 111 1 11111 11 1 11 11 111 1 11111111
C9	1 1 111 11111 11 111111111 1 1111 1 1111 1 11
A1	11111111111111111111111111111111111111111111111111111111111111111111111111111111
A2	11111111111111111111111111111111111111111111111111111111111111111111111111111111
C2	11111111111111111111111111111111111111111111111111111111111111111111111111111111
D3	11111111111111111111111111111111111111111111111111111111111111111111111111111111
D8	11111111111111111111111111111111111111111111111111111111111111111111111111111111
B2	11 1111111111111 1 11 111 11111111111111111111111111 1 1111111
C10	111 1 1111111111 1111 11 1111 111111 11111111111111111111111111
D6	11111111111111 1111 11 1 11 1111111 111111111111
C3	111 1 1 1 1 1 1 111111 1
C11	1 1 1 1 1
C12	11 1 111111111111 1111111111111 1111111 11 11111 1
D7	1 1 1111111111 1 11111 1111111111111 1111111111 1 1 11111
B3	11111 1111111 11111 1 1111 11 1111111 111111 111111111 11111
D2	11 1 1 1 11 11 11 11
E3	11 1 1 1 11111 111111 111 11
B1	111 1 11111111 11111
E1	11 11 11
C8	11 1 1 11 11
D9	1 111 1 1
C7	1 1 1
D5	11 1111 1
E4	11 11 111 111111111111 111 11

ments C1, C4–C6, D1, D4 and E2. The data matrix given in Table 2 shows that genotypes were monomorphic for five bands.

A scatter diagram with DECORANA values along the first two axes is illustrated in Fig. 3. Generally only those DECORANA axes which have eigenvalues greater than 0.1 should be used for interpretation (Hill 1979 b). Eigenvalues on the first and second axes were 0.130 and 0.111, respectively, and were relatively greater than those of the remaining axes that had values lower than 0.1 and therefore not included in the study. DECORANA scores for DNA fragments on these two axes are presented in Table 3. Results from this ordination were again similar to those obtained after the two hierarchical classifications, as genotypes not collected on La Palma gave low scores on the first axis and tended to cluster in the left sector of the scatter diagram (Fig. 3). Furthermore, those DNA fragments responsible for the first major division from the TWINSPAN analysis also had the lowest and highest DECORANA scores on the first ordination axis.

Genotypes from the two accessions of wild tagasaste included in this study did not form distinct groups and were not found separated from the other genotypes in the ordination scatter. There were no clear differences between the variation patterns of germ plasm from these accessions and those from the cultivated populations.

Discussion

A brief initial screen of some of the tagasaste material used in this study showed that the use of a single selected primer would reveal a large amount of variation. When individuals from 20 accessions were used, the primer di-

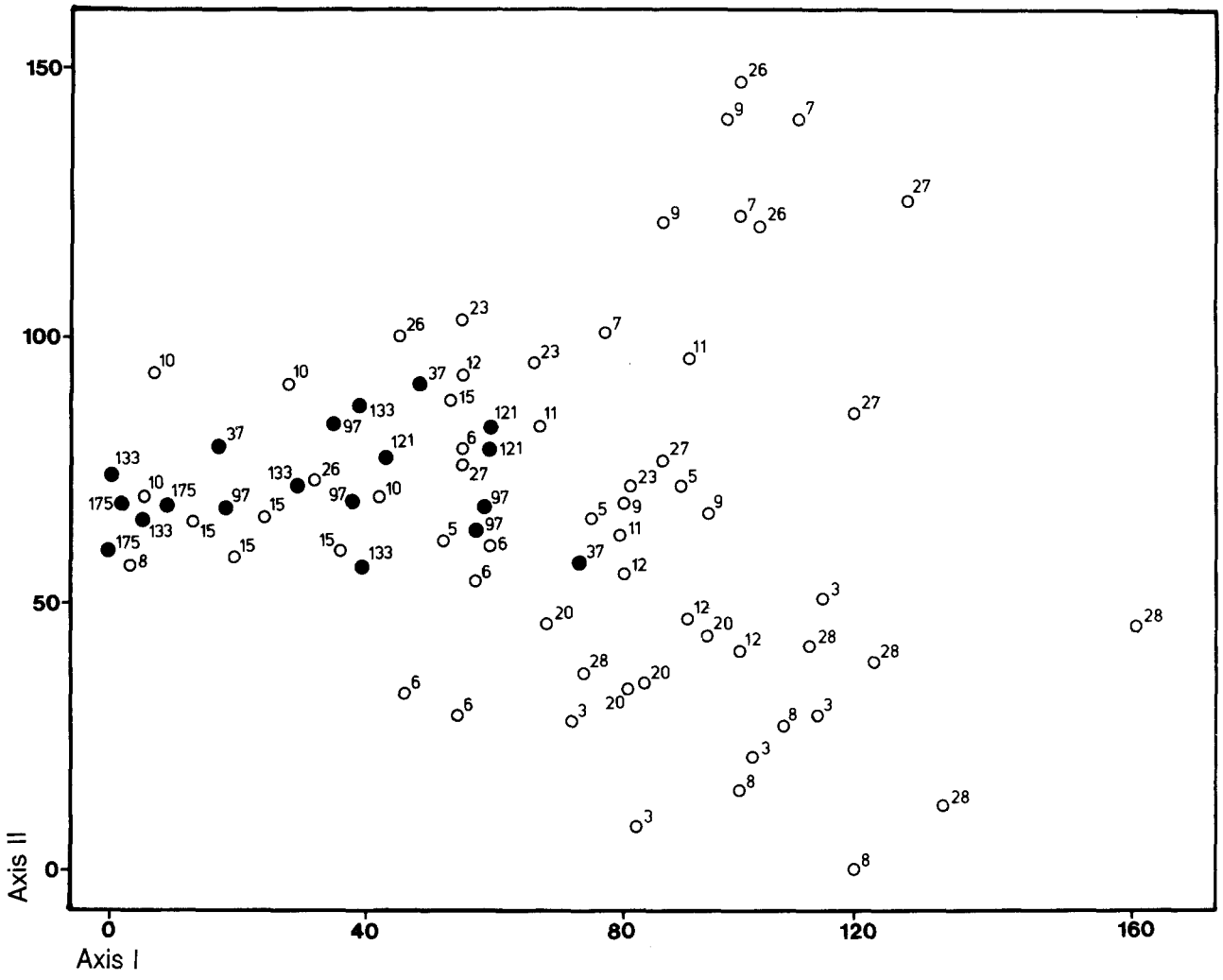

Fig. 3. The first two axes of the DECORANA ordination of 20 accessions of tagasaste. *Labels* refer to accession numbers. Accessions from La Palma are indicated by *open circles*; *closed circles* refer to accessions that were not collected on La Palma

rected the synthesis of amplification products of 30 different sizes that were present in 79 combinations. Because these data were quite sufficient to allow the application of several types of numerical analysis, no further primers were employed.

Patterns of variation discernible after RAPD studies provide evidence which confirms the historical accounts and resultant hypothesis that tagasaste comes originally from La Palma. The multivariate techniques used in this study show that germ plasm collected on La Palma shows by far the widest range of variation compared to that from the other islands. Genotypes from La Palma were found in most of the clusters obtained after TWINSPAN, and this was confirmed by results not reported using cluster analysis (Unweighted pair Group Average Method, Jaccard's coefficient of similarity, Clustan 3, Wishart 1987). Furthermore, those genotypes displayed both high and low scores on the first two DECORANA axes. The fact that genotypes from El Hierro, Tenerife and Gran Canaria all show similar restricted variation supports the view that they have a common origin. This would mean that material originally distributed from La Palma to Tenerife would have formed the basis for the cultivated material found on the other islands.

In terms of the plant genetic resources of tagasaste, the results from RAPD analysis emphasize the importance of conserving germ plasm collected on La Palma; this germ plasm should have the highest priority for conservation and evaluation in contrast to that available on the other islands. Massive deforestation and accidental colonization by non-native mammals such as rabbits and goats have meant that wild plants of tagasaste have become rare on La Palma (Francisco-Ortega et al. 1992b). Consequently, germ plasm conservation should be focused towards those few remaining areas of the laurel (*Laurus azorica* (Seub.) Franco) wood, heath (*Erica arborea* L.) belt and low altitude Canary pine (*Pinus*

Table 3. Ranked values along the first two DECORANA axes for DNA fragments detected after the ordination of 20 accessions of tagasaste

DECORANA 1		DECORANA 2	
DNA fragment	DECORANA value	DNA fragment	DECORANA value
C7	421	E1	452
D9	337	D9	347
D5	325	E2	294
E1	258	D4	206
E4	238	C11	197
B1	234	D6	190
C11	232	C1	183
C8	222	B1	168
C3	197	E3	163
C12	190	C5	129
D7	186	C2	92
E3	177	A1	87
B3	171	A2	87
D6	160	B2	87
A1	53	C10	87
A2	53	D3	87
B2	53	D8	87
C10	53	C4	85
D3	53	D1	78
D8	53	D7	68
C2	51	B3	8
E2	25	D5	−29
D4	−2	C6	−40
D2	−12	C8	−43
C6	−19	C9	−89
C9	−37	C12	−94
C4	−54	E4	−139
C1	−55	D2	−171
D1	−72	C3	−249
C5	−348	C7	−266

canariensis Chr. Sm. ex Dc.) forest of northern La Palma where wild plants of tagasaste still thrive on almost inaccessible cliffs (Francisco-Ortega et al. 1992b).

The scarcity of wild populations of tagasaste has meant that in this research only two accessions of wild tagasaste were studied. However, our techniques did not allow us to distinguish between wild and cultivated germ plasm. Unlike other cultivated species, most fodder crops do not exhibit large differences between wild and cultivated forms, so that the latter may not be regarded as domesticated in the strict sense of the word (Harlan 1983). RAPD analysis and morphology studies (Francisco-Ortega 1992a–c) support the view that tagasaste cannot be considered to be a truly domesticated species.

There are several advantages in the use of the multivariate techniques that we have employed. TWINSPAN is a polythetic divisive algorithm of classification which can identify those key DNA fragments that contribute to each division of the dendrogram. This classification also provides a rearrangement of the Genotype-by-DNA Fragment Data Matrix based on multivariate classifications of both genotypes and DNA fragments. Although caution as to its use has been recommended (Jackson and Somers 1991), DECORANA is an ordination technique which avoids the distortion known as the 'horseshoe' effect that occurs when other methods such as Principal Component Analysis, Reciprocal Average or Non-metrical Multidimensional Scaling are used, and it remains advantageous for treating binary data (Gauch 1982).

Furthermore, TWINSPAN, by arranging the original raw data in a re-ordered Genotype-by-DNA Fragment Data Matrix, facilitates the visual re-interpretation of the initial results and provides a 'bandmap' of shared DNA fragments (Table 2). The use of this kind of bandmap has previously been suggested by Powell et al. (1991) and Wilde et al. (1992) for the identification of cultivars of *Solanum tuberosum* and *Theobroma cacao*, respectively. In the bandmaps suggested by these authors, DNA fragments are entered according to their relative frequencies: the most common fragments being placed first and the least frequent being placed last. In contrast with that, the arrangement of the original raw data from our methodology is based on the emphasis of those DNA fragments that contribute most to the TWINSPAN classification. We therefore recommend the use of TWINSPAN because both DNA fragments and genotypes are entered in the bandmap according to their effectiveness in the classification.

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References

Acebes-Ginovés JR, Del Arco-Aguilar M, Wildpret de la Torre W (1991) Revisión taxonómica del género *Chamaecytisus* Link en Canarias. Vieraea 20:191–202

Francisco-Ortega J (1992) An ecogeographical study within *Chamaecytisus proliferus* (L. fil.) Link complex (Fabaceae: Genisteae) in the Canary Islands. PhD thesis, The University of Birmingham, UK

Francisco-Ortega J, Jackson MT, Santos-Guerra A, Fernández-Galván M (1990) Genetic resources of the fodder legumes tagasaste and escobon (*Chamaecytisus proliferus* (L. fil.) Link *sensu lato*) in the Canary Islands. FAO/IBPGR Plant Genet Resources Newsl 81/82:27–32

Francisco-Ortega J, Jackson MT, Santos-Guerra A, Fernández-Galván M (1991) Historical aspects of the origin and distribution of tagasaste (*Chamaecytisus proliferus* (L. fil.) Link ssp. *palmensis* (Christ) Kunkel), a fodder tree from the Canary Islands. J Adelaide Bot Gard 14:67–76

Francisco-Ortega J, Jackson MT, Socorro-Monzón AR, Ford-Lloyd BV (1992a) Ecogeographical characterization of germ plasm of tagasaste and escobon (*Chamaecytisus proliferus*

(L. fil.) Link *sensu lato*) from the Canary Islands: soil, climatological and geographical features. Invest Agric Prod Veg (in press)

Francisco-Ortega J, Jackson MT, Santos-Guerra A, Fernández-Galván M, Ford-Lloyd, BV (1992b) The phytogeography of the *Chamaecytisus proliferus* (L. fil.) Link (Fabaceae: Genisteae) complex in the Canary Islands: a multivariate analysis. Vegetatio (in press)

Francisco-Ortega J, Newbury, HJ, Ford-Lloyd BV, Soriano O (1992c) Tagasaste in the Canary Islands in the 17th century. J Adelaide Bot Gard 7(3):377–388

Gauch GG (1982) Multivariate analysis in community ecology. Cambridge University Press, Cambridge

Gawel NJ, Jarret RL (1991) A modified CTAB DNA extraction procedure for *Musa* and *Ipomea*. Plant Mol Biol Rep 9:262–266

Harlan JR (1983) The scope for collection and improvement of forage plants. In: McIvor JG, Bray RA (eds) Genetic resources of forage plants. Commonwealth Science and Industrial Research Organization, East Melbourne, pp 3–14

Hill MO (1979a) Twinspan a fortran program for arranging multivariate data in an ordered two-way table by classification of the individuals and attributes. Section of Ecology and Systematics, Cornell University, New York

Hill MO (1979b) Decorana a fortran program for detrended correspondence analysis and reciprocal averaging. Section of Ecology and Systematics, Cornell University, New York

Jackson DA, Somers KM (1991) Putting things in order; the ups and downs of detrended correspondence analysis. Am Nat 137:704–712

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Mohler CL (1987) Cornell ecology programs ms-dos microcomputer package. Microcomputer Power, Ithaca, New York

Morris D (1893) Tagasaste (*Cytisus prolifer* L. var 'palmensis' Chr.). Kew Gard Bull Misc Inf 1893:115–117

Newbury HJ, Ford-Lloyd BV (1993) The use of RAPD for assessing variation in plants. Plant Growth Reg 12:43–51

Pérez V (1862a) Agricultura. El Guanche (Santa Cruz de Tenerife) 294

Pérez V (1862b) Tagasaste o escobón de La Palma. El Guanche (Santa Cruz de Tenerife) 299

Pérez-Ventoso JV (1892) Le tagasaste (*Cytisus proliferus* varietas) fourrage important. Semaine Medicale, Paris

Powell W, Phillips MS, McNicol JW, Waugh R (1991) The use of DNA markers to estimate the extent and nature of genetic variability in *Solanum tuberosum* cultivars. Ann Appl Biol 118:423–432

Reghunath BR, Francisco-Ortega J, Newbury HJ, Ford-Lloyd BV (1993) Methods for increasing the efficiency of seed germination in the fodder legumes tagasaste and escobon (*Chamaecytisus proliferus* (L. fil.) Link *sensu lato*). Seed Sci Technol 21:225–235

Wilde J, Waugh R, Powell W (1992) Genetic fingerprinting of *Theobroma* clones using randomly amplified polymorphic DNA markers. Theor Appl Genet 83:871–877

Williams JG, Kubelik AR, Livak KJ, Rafalski LA, Tingey SV (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Wishart D (1987) CLUSTAN, user manual, 4th edn. University of St. Andrews, St. Andrews, Scotland