A CLASSIFICATION OF TRADITIONAL RICE VARIETIES (*ORYZA SATIVA* L.) FROM AFRICA USING ISOZYMIC VARIABILITY

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Abstract

Over 700 traditional varieties of rice collected from 12 African countries were analysed for their isozymic variability. Multivariate analysis performed on the 232 genotypes identified enables a classification to be proposed. The two main types (*indica* and *japonica*), easily separable by the phenol reaction, were identified and are well differentiated which suggests that environmental conditions and rice cultivation methods may exercise selective pressures favouring parental genotypes. Two major *japonica* and five *indica* subgroups are described.

Introduction

Following the work of Kato (1930), the cultivated Asian species of rice, *Oryza sativa* L., is divided into two main types, *indica* and *japonica*. Various criteria have been used in order to describe these two groups; for example, morphophysiological characters (Matsuo, 1956; Oka, 1958; Chang and Bardenas, 1965; Morishima and Oka, 1982), genetic characters (Oka, 1958, 1983; Second, 1982), biochemical characters (Oka, 1958; Nakagahra, 1978; Endo and Morishima, 1983; Second, 1982, 1984; Glaszmann, 1982) and others. . . (see Second, 1984, for a review).

Using electrophoresis as a classificatory tool, Chu (1967), Shahi *et al.* (1969), Pai *et al.* (1973) and Fu and Pai (1979) identified peroxidase alleles specific to the *indica* and *japonica* types. Pai *et al.* (1975), Fu and Pai (1979) obtained similar results for acid phosphatase alleles. Nakagahra *et al.* (1975) and Nakagahra (1977) proposed a system of classification based on three esterase loci. A more complete analysis was made by Glaszmann *et al.* (1984) which demonstrated a multiallelic specificity within the two types.

Following the hypothesis of Second (1982), according to which the *indica* and *japonica* types originate from two distinct centres of domestication, we will consider them as two subspecies.

All previous studies were conducted with Asian varieties or with a very few varieties from elsewhere but *O. sativa* is also a staple crop in many parts of Africa, where its variability has been found to be at least equal to that found in Asia (de Kochko, 1987). A better knowledge and understanding of the status of rice and its varietal distribution in Africa is a prerequisite to rice improvement within the continent.

In this paper we describe the variability of numerous loci in order to identify multiallelic associations among samples of traditional African rice varieties collected from several countries. A classification is proposed and the geographical distribution of the groups identified is given.

Materials and methods

Plant material

Over 700 rice varieties from the ORSTOM collection, maintained in the Ivory Coast and originating from 12 African countries, were analysed. Only those showing different genotypes for 17 polymorphic loci representing 37 frequent and 8 minor alleles, totalling 232 genotypes, were used in the multivariate analyses.

Electrophoresis

The enzymatic systems analysed are presented in Table 1. All the extraction, running and staining procedures used are described in Second and Trouslot (1980) and de Kochko (1987). Interpretation of the zymograms are also given in these papers.

Multivariate analyses

For each locus, each of the alleles encountered was designated as a variable; if present it was scored 1, if absent it was scored 0. After the construction of a Burt Table, all the genotypes were submitted to a Factorial Component Analysis (FCA) and a Hierarchical Classification (HC).

The computer programs used were taken from the Statistical Package for Social Science (SPSS) and the 'Association pour le Developpement de l'Analyse des Données (ADDAD). They are kept by the 'Centre Universitaire du Traitement de l'Information' (CUTI) of the National University of the Ivory Coast. The programs

Table 1. Enzyme systems analysed

Enzyme	Abbreviation	Reference
Oxydo-reductases:		
Peroxidase	POX	Second and Trouslot, 1980
Catalase	CAT	Second and Trouslot, 1980
Alcool Dehydrogenase	Adh	Second and Trouslot, 1980
Glutamate Dehydrogenase	Gdh	Second and Trouslot, 1980
Malate Dehydrogenase	Mdh	Second and Trouslot, 1980
Isocitrate Dehydrogenase	ICD	Second and Trouslot, 1980
Phosphogluconate Dehydrogenase	PGD	Second and Trouslot, 1980
Shikimate Dehydrogenase	SKdh	Weeden and Gottlieb, (cited by Tanksley and Rick, 1980)
Transferases:		
Glutamateoxaloacetate Transaminase	GOT	Second and Trouslot, 1980
Phosphogluco Mutase	PGM	Second and Trouslot, 1980
Isomerase:		
Phosphogluco Isomerase	PGI	Second and Trouslot, 1980
Hydrolases:		
Esterase	EST	Second and Trouslot, 1980
Leucine Amino Peptidase	LAP	Second and Trouslot, 1980
Acid Phosphatase	ACP	Second and Trouslot, 1980
Endopeptidase	EP	Cardy *et al.*, 1981

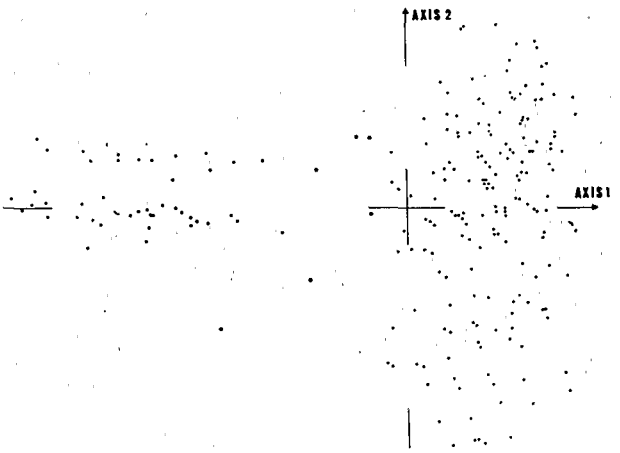

Fig. 1. Projection of the individuals along axes 1 and 2 of the first FCA, performed with all the genotypes.

were run on an IBM 43-41 computer at the 'Office Central de la Mécanographie'.

Phenol reaction

As mentioned by Oka (1958), the phenol reaction is a good criterion to discriminate between the two types, *indica* and *japonica*, and it is equally effective with African rice (de Kochko, 1987). All the varieties were tested for their response to the phenol reaction and the results were related to their multiallelic associations. The grains were soaked in a 2% phenol solution for 48 h. The change in colour of the hull was compared with that of grains soaked in distilled water for the same period.

Results

A Factorial Component Analysis (FCA) was performed on the 232 genotypes encountered. It shows a separation between two main groups, labelled I and II. Fig. 1 indicates that this separation occurs on the first axis and represents 67.6% of the total variability.

Table 2. Allelic distribution between the two main groups. A: Alleles specific group I. B: Common alleles. C: Alleles specific to group II.

A	B		C
			EST.Ca1
			EST.B0
		EST.D1	EST.C2
	EST.Ca2	EST.E0	EST.E2
EST.D0	EST.B1	EST.E1	POX.B4
POX.C2	EST.C0	POX.C1	LAP.E2
CAT.A2	POX.B3	LAP.E1	PGI.A1
ICD.A2	PGI.A2	LAP.E3	PGI.B2
ACP.Amc+9	PGI.B1	PGD.A1	PGD.A2
ACP.Fa0	SKdh.A4	PGD.A3	SKdh.A3
	EP.A4	CAT.A1	SKdh.A5
		ICD.A1	EP.A0
			ACP.Amc−4
			ACP.Fa+

Group I is distributed along negative values of axis 1. Group II is located towards the positive values of this axis and is distributed along axis 2 which in turn accounts for 6.4% of the variability. Very few intermediates are located between the two groups.

A Hierarchical Classification (HC) performed on the factorial variables gives a better visualisation of the groups and demonstrates the associations between the groups and the alleles that defined them.

Table 2 shows the allelic distribution, where each group is characterised by a few alleles. Comparison with the results published by Second (1982, 1984) and Glaszmann

Table 3. Allelic composition of each group. Alleles are presented in order of importance. Alleles in parentheses are rare ($f < 0.05$); those separated by '/' are equally frequent.

Locus	Ia	Ib	Ic	IIa	IIb	IIc	IId	IIe	IIf
EST.Ca	2-(1)	2	2	1	1-2	1-2	1	2-(1)	1-(2)
EST.B	1	1	1	1	1-(0)	1	1-0	1-(0)	1
EST.C	0-(2)	0-(2)	0	2-0	2-0	2-0	2-0	2-0	2-0
EST.D	0/1	0-1	0-1	1	1	0	1	1	1
EST.E	0-(1)	1-0	1-0	1-2	0-2-(1)	2-1	1/2	2-1	2-1
POX.B	3-(4)	3	3	3-4	3-4	3-4	4-3	4-3	4-3
POX.C	1-2	1-2	1-2	1-(2)	1	1	1	1	1-(2)
LAP.E	1-(3)	1	1	2-1-(3)	1-2-3	1-2	1	1-2	1
PGI.A	2	2	2	1-2	1-(2)	1-2	1-2	1-2	1-2
PGI.B	1	1	1	1-(2)	1-(2)	1-2	2-1	1-2	1-2
PGD.A	1	3-1	1-3	1-2-(3)	1-2-(3)	3-1	1-3	1-3	1-3-2
CAT.A	1-2	2-1	2-1	1	1	1	1	1	1
ICD.A	1	1	2	1	1	1	1	1	1
SDH.A.	4	4	4	3-4	4-3	4-3	4-3	3-4	3-4-5
EP.A	4-(3)	4	4	4	4-(0)	4	4-(0)	4-0	4
ACP.A	±9	±9	±9	-4	-4	-4	-4	-4,(+9)	-4,(+9)
ACP.Fa	0	0	0	+	+-(0)	+	+-(0)	+-(0)	+-(0)
PHENOL	-;(+)	-;(+)	−	+;(-)	+	+	+	+;(-)	+

et al. (1984) enables us to relate group I to the *japonica* type and group II to the *indica* type.

Japonica varieties have only six characteristic markers exclusive to this group (with a very few exceptions). 18 alleles are common to the two groups and 14 are characteristic of the *indica* group. In addition, nearly all the varieties from group I are phenol negative while those of group II are phenol positive.

The HC subdivides the *japonica* genotypes into two major subgroups and a minor one (Ia, Ib and Ic). The *indica* genotypes split into several subgroups.

Subgroups Ia and Ib are distinguished by the frequencies at the EST.E locus (Table 4). Furthermore, all the varieties from subgroup Ib carrying the EST.E0 allele have the PGD.A3 allele in association; this allele is absent from subgroup Ia.

In order to examine the variability within *indica* more closely, the group II data only were reanalysed by a further FCA and HC. Five subgroups were then identified. Fig. 2 shows the inertia elipses of these subgroups along axes 1 and 4. Subgroup IId, which appears superimposed on subgroups IIb and IIf, is well separated from them along axes 1 and 2 (figure not shown). This separation is explained below.

Table 3 gives the allelic composition of each group which, considered together with the results in Table 4, shows the related allelic frequencies and makes it possible to identify the allelic structure of each subgroup. Subgroups Ic and IIc are each defined by one rare allele, respectively ICD.A2 and EST.D0. The latter allele is characteristic, in Africa, of the non-*indica* varieties.

Euclidian distances between seven of the nine subgroups were calculated on the basis of the allelic

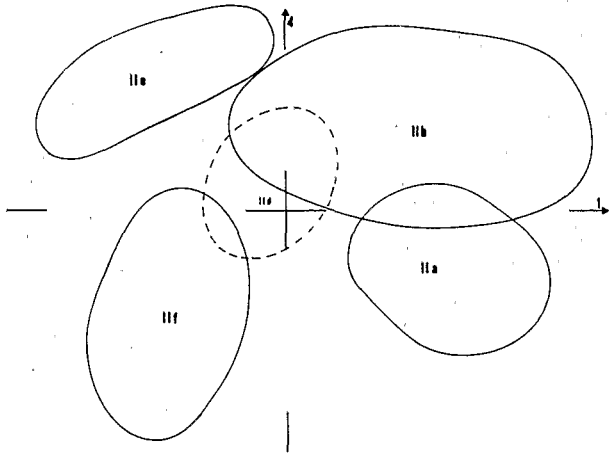

Fig. 2. Projection of the inertia elipses of the sub-indica groups along axes 1 and 4 of the second FCA, performed only with *indica* genotypes.

frequencies (subgroups Ic and IIc were not considered because of their small size). Fig. 3 shows the dendrogram obtained. Subgroups Ia and Ib, IIa and IIb, IIe and IIf are very close to each other, subgroup IId is rather more remote while, as expected, subgroups Ia and Ib are distant from the remaining five.

The distribution of the different subgroups is not equal in all countries. As shown in Table 5, the Madagascan varieties fall within four subgroups (Ia, Ib, IIa and IIb). Varieties from the Ivory Coast include only three subgroups (except one variety that falls within subgroup IIc) of which subgroup Ic is recorded only from there. The samples from Guinea Conakry show the widest range but a large proportion of them (44%) fall within one

subgroup (IIf). The Guinea Bissau material is also well distributed between the different subgroups. For the others countries it is interesting to note that in the Sahelian region no varieties from subgroups Ia and Ib were found. All Tanzanian varieties that were classified in these two subgroups were from the islands of Zanzibar and Pemba. Subgroup IId is important only in East Africa.

Discussion

Isozymic variability within African rice varieties enables the identification of two main groups which can, due to their multiallelic associations, be considered as belonging to the *japonica* and *indica* types recognised in Asia. Multivariate analyses carried out on the African genotypes permit a more detailed classification to be proposed.

The *japonica* type is divided into two major subgroups. There does not seem to be any relationship between these subgroups and their geographical distributions.

The intermediate type defined by Nakagahra *et al.* (1975) is characterised by the association EST.D0–EST.E1 ($Est1^0$, $Est2^1$) and it may therefore be equivalent to our subgroup Ib which shows a predom-

Table 4. Allelic frequencies in each group in relation to the number of plants. Groups Ic and IIc are not represented because of their small size.
*Other rare alleles

Allele		Ia	Ib	IIa	IIb	IId	IIe	IIf
EST.Ca	1	<0.01	0	1	0.89	1	0.04	0.98
	2	>0.99	1	0	0.11	0	0.96	0.02
EST.B	0	0	0	0	0.02	0.47	0.04	0
	1	1	1	1	0.98	0.53	0.096	1
EST.C	0	0.98	0.97	0.29	0.46	0.32	0.41	0.31
	2	0.02	0.03	0.71	0.54	0.68	0.59	0.69
EST.D	0	0.50	0.79	0	0	0	0	0
	1	0.50	0.21	1	1	1	1	1
	0	0.99	0.19	0	0.89	0	0	0
EST.E	1	0.01	0.81	0.64	0.01	0.50	0.46	0.16
	2	0	0	0.36	0.09	0.50	0.54	0.84
POX.B	3	>0.99	1	0.74	0.79	0.45	0.44	0.19
	4	<0.01	0	0.26	0.21	0.55	0.56	0.81
	1	0.88	0.96	0.97	1	0.97	1	0.98
POX.C	2	0.09	0.04	0.01	0	0	0	0.02
	*	0.02	<0.01	0.01	0	0.03	0	0
	0	0	<0.01	0	0	0	0	0
LAP.E	1	0.98	>0.99	0.31	0.74	1	0.89	1
	2	0	0	0.68	0.14	0	0.11	0
	3	0.02	0	0.01	0.12	0	0	0
PGI.A	1	0	0	0.78	0.98	0.84	0.57	0.63
	2	1	1	0.22	0.02	0.16	0.43	0.37
	1	1	1	0.97	0.98	0.29	0.78	0.88
PGI.B	2	0	0	0.03	0.02	0.68	0.11	0.12
	*	0	0	0	0	0.03	0.11	0
	1	1	0.48	0.67	0.69	0.61	0.59	0.46
PGD.A	2	0	0	0.29	0.28	0	0	0.14
	3	0	0.52	0.04	0.03	0.39	0.41	0.40
CAT.A	1	0.86	0.50	1	1	1	1	1
	2	0.14	0.50	0	0	0	0	0
	3	0	0	0.73	0.15	0.23	0.81	0.83
SKdh.A	4	1	1	0.27	0.85	0.77	0.19	0.10
	5	0	0	0	0	0	0	0.07
	0	0	0	0	0.02	0.24	0.04	0
EP.A	3	<0.01	0	0	0	0	0	0
	4	>0.99	1	1	0.98	0.76	0.96	1
ACP.Amc	9	1	1	0	0	0	0.02	0.02
	−4	0	0	1	1	1	0.98	0.98
ACP.Fa	+9	1	1	0	0.02	0.03	0.02	0.03
	+	0	0	1	0.98	0.97	0.98	0.97
PHENOL	−	>0.99	>0.99	0.02	0	0	0.05	0
	+	<0.01	<0.01	0.98	1	1	0.95	1
SIZE		138	191	73	85	38	54	109

inance of these two alleles. In the same way, our subgroup Ia may be compared to the *japonica* type of these authors, characterised by the association EST.D1−EST.E0 ($Est1^1Est2^0$) as EST.E0 is almost the only allele at the Est.E locus and Est.D1 is present in half of the varieties classified in this subgroup.

Glaszmann *et al.* (1984) did not propose subdivisions of their two groups; they called the group in which they classified the *japonica* and *javanica* (tropical *japonica*) varieties 'not-*indica*'. Comparison of their 'not-*indica*' group and the present group I shows a close correspondence. The same is true for the *indica* group in both cases.

In addition, these authors found that the upland rices from Africa could be classified within the group 'not-*indica*', which is also in close agreement with results obtained here considering African cultivars only. The third *japonica* subgroup found in this study is peculiar to the Ivory Coast and is characterised by presence of the rare allele ICD.A2.

Diversification among the *indica* type is greater as five

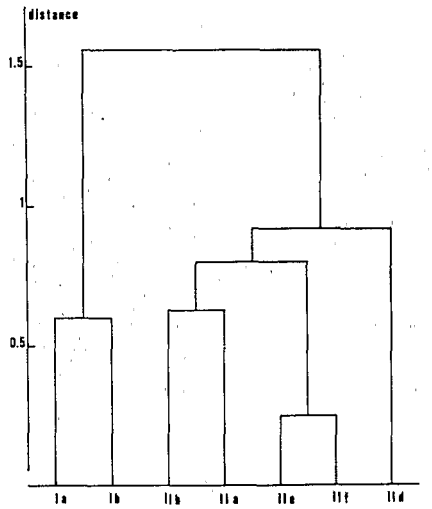

Fig. 3. Euclidian distances between 7 of the 9 subgroups. Subgroups Ic and IIc are not considered because of their small size.

major subgroups may be defined. The majority of the varieties from each country or region belong to a particular subgroup. These include subgroup IIb from Madagascar, IIf from Guinea Conakry, IIf and IIe from Guinea Bissau, IIa from Cameroon, Chad and Nigeria, IIf from Sahelian countries and IId from East Africa.

The subgroup IIb, the majority of which comprises *indica* from Madagascar, is characterized by the EST.E0 allele that was defined by Nakagahra (1977) and Second (1982) as characteristic of *japonica*. The varieties carrying this allele were mainly collected in the high plateau region where the environment probably exerts a selective pressure in favour of these intersubspecific recombinants that may have evolved locally as both *indica* and *japonica* types are present, or could have been introduced with such a genome already present. As a matter of interest, the plateau region is populated by Indo-Malaysian immigrants who, on their voyage from their region of origin, certainly stopped in Sri Lanka where the *indica* varieties often carry the allele EST.E0 (Glaszmann *et al.*, 1984).

The phenol reaction is very effective for discriminating between the African varieties as nearly all from group I give a negative response while those from group II give a positive result. This test, easy to perform, can be used to detect quickly and accurately the group to which an unknown traditional cultivar belongs.

It is surprising that in Africa there is a differentiation between the two main groups of rice varieties which mirrors exactly the situation found in Asia.

It has already been shown that Africa contains a genetic diversity of rice as important as that found in Asia (de Kochko, 1987). Since the introduction of rice in this new environment one would expect a mixing by means of natural intersubspecific crosses, as indeed is known to be the case since the two types are often cultivated together in the same region (Madagascar, Guinea, Tanzania), and electrophoresis reveals the existence of some heterozygotes (<2%). However, it seems that in addition to the selective barriers and hybrid weakness which promote a return to parental forms (Oka, 1964), environmental conditions and particularly the methods of

Table 5. Geographical distribution of the group. C.T.Na: Cameroon (north), Chad (south), Nigeria (north). B.M.Nr: Burkina Faso, Mali, Niger.

Country	Ia	Ib	Ic	IIa	IIb	IIc	IId	IIe	IIf
MADAGASCAR	0.23	0.21	–	0.16	0.29	<0.01	<0.01	0.06	0.03
IVORY COAST	0.61	0.33	0.06	–	–	–	–	<0.01	–
GUINEA CONAKRY	0.05	0.13	–	0.07	0.07	0.02	0.07	0.15	0.44
BISSAU GUINEA	0.10	0.19	–	0.10	0.05	–	0.10	0.24	0.24
C.T.Na	0.09	–	–	0.41	0.14	–	0.09	–	0.27
B.M.Nr	–	–	–	–	0.22	0.09	0.04	0.22	0.43
TANZANIA	0.17	0.03	–	0.20	–	–	0.43	0.03	0.13
ZAMBIA	–	–	–	0.35	0.12	–	0.47	–	0.06

rice cultivation exercise selective pressures that favour certain genotypes which are very close to the parental ones.

Exceptions are the highland Madagascan cultivars which have genotypes quite distinct from the parental ones. Whether these are native or introduced, the local environment certainly favours their existence.

It is hoped that the classification proposed will help plant-breeders in establishing breeding programmes using intra-subspecific crosses (intra group I or II) as well as inter-subspecific ones (between group I and II).

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