

# Asian Rice

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Rice is part of the heritage of the most ancient civilizations—in China, close to the Yangtze river, traces of rice cultivation date to about seven millennia ago. Today, in the developing countries, rice represents nearly 50% of the cereal production, far more than wheat and maize. The world production of paddy, or dehulled rice, is close to 540 million t. It is cultivated on about 150 million ha, of which 90% is in Asia. In this region, the per capita annual consumption of milled rice is often higher than 100 kg, and can reach up to 200 kg in Myanmar or Laos. On other continents, the consumption is more variable but can reach 100 kg, particularly in Madagascar, Sierra Leone, or Surinam. In the European Union, the per capita consumption of rice is about 5 kg. Most rice is consumed locally, international commercial trading amounting to less than 5% of the production. However, with economic progress, the populations that are the highest consumers of rice are gradually diversifying their diet, while populations that consume small amounts are progressively integrating more rice into their diet. According to demographic forecasts, by the year 2025 the demand for rice is likely to increase by 70%.

Rice is a semi-aquatic plant: it tolerates aquatic cultivation conditions but does not absolutely depend on them. Since the beginning, development of rice cultivation has been dependent on a sufficient natural fertility of the soil and on a low weed competition. The most favourable situations are found in flooded lands and recently cleared forest lands.

Cultivation on flooded lands has developed most, particularly because of improvements in irrigation and drainage and the possibility of growing rice continuously. Rice is direct-seeded or transplanted from nurseries. The wet lowlands of valleys and wide plains are systematically exploited. Deep-water rice cultivation is practised in areas subject to floods of 4 to 5 m in Bangladesh and Mali, using varieties that can elongate their stems by several centimetres a day. Close to the coastal areas, soil desalination techniques using fresh water have been developed to grow one rice crop, for example in

Indonesia and Guinea. Inland, in hilly areas, terrace cultivation is practised, as in Nepal, the Philippines, and Bali. Aquatic rice cultivation is found thus from the equator to latitudes of 40°N, even 50°N in China, and in the tropical regions from sea level to over 2000 m altitude in Nepal and Madagascar notably.

In the humid tropical regions, apart from conditions of aquatic cultivation, upland rice cultivation depending exclusively on rainfall for its water requirement is also practised. It is first developed on cleared forest land. After slashing and burning of the forest, rice is broadcast or dibbled. The cultivation requires little input in the first two or three years, then the natural fertility of the soil decreases while weeds begin to invade the area. Then the cultivation area is shifted. This practice gradually disappears with increasing demographic pressure. Other cropping systems are found in Brazil, where upland rice is cultivated on millions of hectares before the installation of pastures, as well as in several countries where upland rice is intercropped with young plantations of rubber tree, coffee, or other perennial crops.

## TAXONOMY AND GENETIC RESOURCES

### Cultivated Rice Within the Genus *Oryza*

Cultivated rice belongs to the genus *Oryza*, which is highly diverse, and comprises several genomes recognized on a cytogenetic basis, as well as diploid and allotetraploid forms. More than 20 wild species are recognized, of which 9 are tetraploid. They are now distributed on all the continents but the origin of the genus is probably Asia (Second, 1985).

Two species are cultivated: one is of African origin and found almost exclusively in West Africa, and the other is of Asian origin. Both are diploid and autogamous. The African cultivated species, *O. glaberrima*, results from domestication of *O. breviligulata* in Africa. The Asian cultivated species, *O. sativa*, has an original genetic structure, which is the focus of the work described in this chapter. Its domestication, from the species *O. rufipogon*, is ancient: remains of grains associated with human activities almost 4500 years old have been found in Pakistan, some dating from over 8500 years ago in northern India, some dating from at least 6000 years ago in Thailand, and some dating from 7000 years ago in China. There have been constant migrations in a larger circle towards the west to Pakistan and Iran, towards the east—rice was introduced in Japan from the beginning of the first century—and towards the southern islands of Sri Lanka, Malaysia, the Philippines, Indonesia, and Taiwan. Malay navigators probably introduced rice from Indonesia to Madagascar in the 5<sup>th</sup> or 6<sup>th</sup> century. Much later, Europeans introduced Asian rice in tropical Africa and then in America.

Cultivated rices have a wide diversity of forms. Geneticists, notably in Japan and India, attempted very early to classify the varieties to summarize

the global variability into a few subgroups. To do so, they used morphological and physiological characters and observed the crossing behaviour. Two subspecies were soon identified, *indica* and *japonica*, with 'temperate' and 'tropical' types for the latter (Oka, 1958). Some breeders distinguished three morphological types, Indica, Japonica, and Javanica (Chang, 1976), this last type being variously positioned.

## Genetic Resources

The genetic resources of rice have received a great deal of attention, notably because of the existence of the International Rice Research Institute (IRRI), focussing strictly on rice (Chang et al., 1984). From the 1930s to the 1950s, the major rice-producing countries in Asia built up important national germplasm banks: the germplasm bank in India comprised over 20,000 accessions, and the one in China close to 40,000 accessions. The IRRI, established in 1961, rapidly assumed a leading role, serving as a centre for international seed exchange and for medium-term conservation thanks to high-quality facilities. The spread of semi-dwarf, high-yielding varieties subsequently stimulated international collaboration in the task of collection. Financial support from another international organization, the IBPGR (International Board for Plant Genetic Resources), facilitated this collaboration. Between 1971 and 1982, more than 25,000 samples were collected. French institutions participated by supporting collections in Africa. Since 1994, a programme of complementary collection in the less represented regions has been financed by the Agence Suisse pour le Développement et la Coopération. Today the international germplasm bank has more than 80,000 accessions, 95% of which belong to the *O. sativa* species (Jackson, 1997).

The international germplasm bank comprises all the samples conserved in some but not all the national germplasm banks. A duplicate of the samples is systematically deposited at the conservation centre at Fort Collins, in the United States, after rejuvenation of the accessions. The African accessions are duplicated at IITA (International Institute for Tropical Agriculture) in Nigeria and at ADRAO (Association pour le Développement de la Riziculture en Afrique de l'Ouest), in Côte d'Ivoire. Besides research on the physiology and conservation of seeds, many studies have been undertaken to characterize and evaluate the accessions for botanical descriptors and characters of agronomic interest (Jackson, 1997).

## ORGANIZATION OF GENETIC DIVERSITY: THE CONTRIBUTION OF MOLECULAR MARKERS

Molecular markers contribute a great deal to our comprehension of the genetic diversity of rice. Isozymes, despite the limited number of loci and alleles

available, provided the primary elements of quantification of intravarietal differentiation. In Asia, the cultivated species seems fundamentally bipolar, which is remarkably consistent with the work of Oka (1958), but with a continuum of intermediate forms (Second, 1982). Most of the traditional varieties can be divided into two major groups, corresponding to the *indica* and *japonica* subspecies. Only 20% of the varieties are not clearly classified into either group (Glaszmann, 1987, 1988). Isozymes have also shown a triangular equidistance between the African cultivated species, the typical *indica* forms, and the typical *japonica* forms (Second, 1982). The concept of a molecular clock was applied to the scenario of paleoenvironmental modifications (Second, 1985) and has enabled an overall interpretation of the genetic structure of the genus *Oryza*, with a scenario of triple domestication from wild species appearing around two million years ago, following the isolation of tropical Africa by the cooling of the climate and the emergence of the Himalayas as a geographic barrier in Asia. A particular form was also discovered in western India; it presents traces of introgression of *O. glaberrima* (Lolo and Second, 1988). The inventory of forms that are not classified into the *indica* and *japonica* subspecies cannot be considered complete.

The classification established on the basis of isozymes globally agrees with the local, empirical classifications as well as with synthetic classifications such as those of Oka (1958) and Cheng et al. (1984), established on multiple criteria associating morphological components such as grain width or hairiness with physiological components such as cold tolerance of seedlings (Zhou et al., 1988).

With the advent of new molecular techniques, studies based on different types of molecular markers have been undertaken, from which different infraspecific classifications can be constructed. Each newly obtained scheme has generally been comparable directly to one, sometimes two or three, of the existing schemes using the material common to the various studies, the more global comparisons being done step by step. Remarkably similar images, which indicate a very marked bipolar structure, have been obtained with the different tools used: isozymes (Second, 1982, 1985; Glaszmann et al., 1984; Glaszmann, 1985, 1987, 1988), RFLP (Wang and Tanksley, 1989; Zhang et al., 1992; Ishii et al., 1995; Second and Ghesquiere, 1995), RAPD (Virk et al., 1995; Mackill, 1995; Parsons et al., 1997), ISSR-PCR (Parsons et al., 1997; Blair et al., 1999), AFLP (Zhu et al., 1998), and microsatellites (Yang et al., 1994), the last giving a hazier image because of its greater polymorphism and numerous rare allelic forms.

Moreover, Virk et al. (1996) indicated the value of molecular markers in predicting characters of agronomic interest with quantitative expression when a sample of highly diverse varieties is considered. This observation carries in itself the question of the organization of the genetic diversity and its control: What structure is associated with what characters? With what mechanisms of maintenance or evolution? And with what uses?

We propose here to compare several classifications based on different types of characters, concentrating the analyses on the common samples (see Appendix). Then, we examine some specific situations that enable us to tackle some aspects of the dynamics that conditions the genetic diversity of cultivated rice.

## Diversity Revealed by Different Types of Markers

### ISOZYMES

The largest study was done on a sample of 1688 traditional Asian varieties characterized for 15 polymorphic loci (Glaszmann, 1987). Six groups were distinguished: two major groups (I and VI) with pan-Asian distribution, two minor groups (II and VI) found essentially on the Himalayan foothills of South Asia, and two particular groups (II and IV) restricted to deep-water zones in Bangladesh. Group I represents the typical *indica* forms and group VI the typical *japonica* forms. Group II contains notably the rices of the 'aus' ecotype of northern India and Bangladesh. Group V is represented by high-quality rices (aroma, elongation on cooking) of the 'basmati' group of India and Pakistan. Groups II and IV correspond to floating rices, among which some, such as the 'rayada' of group IV, are capable of stem elongation to more than 5 m.

A sample A of 270 varieties, constituted to represent the geographic, ecotypic, and enzymatic diversity of cultivated rice of Asian origin (see Appendix), was used for a similar analysis. From the 15 loci, 57 allelic forms could be revealed. These data were subjected to a correspondence analysis (CA). The percentage of variation explained by the CA axes decreased very rapidly: it was 18.7%, 9.2%, 7.9%, 6.3%, and 5.1% for axes 1 to 5 respectively. This rapid decrease illustrates a very marked structure. Figure 1 shows the distribution of accessions in the 1-2 plane of the CA. The six groups, I to VI, are identified in agreement with the results obtained on larger samples.

### RFLP

A sample B of 147 varieties was analysed using 202 probes (see Appendix). Among these, 148, or 74%, revealed a polymorphism and produced a total of 482 polymorphic bands. These data were subjected to a CA. The percentage of variation explained by the CA axes decreased very rapidly: they were 16.5%, 6.2%, 4.4%, 3.8%, and 3.1% for axes 1 to 5, indicating, as for the isozymes, a marked general structure.

Figure 2a shows the distribution of the 147 varieties studied in the 1-2 plane. The position of the varieties on the primary axis is by far the one that characterizes them the best. It is particularly interesting to remark that this axis represents the variation between the reference *indica* varieties (isozymic group I) and the reference *japonica* varieties (isozymic group VI). Groups II,

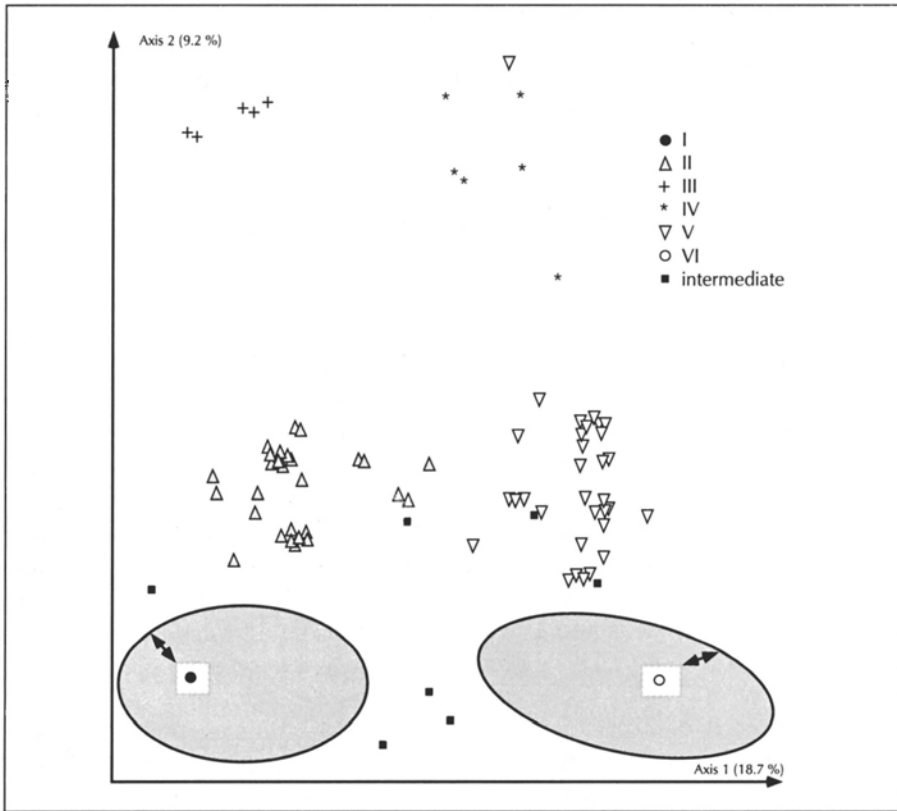


Fig. 1. Distribution on the 1-2 plane of the CA on isozyme data (15 loci and 57 alleles) characterizing sample A.

III, IV, V, and 'intermediates' appear overall in an intermediate position between the reference *indica* and *japonica* varieties, with, however, a low percentage of specific alleles. With this restriction, *O. sativa* appears organized according to a principal axis of *indica-japonica* variation.

In the refined analysis of *O. sativa* diversity, a new result is seen on axis 3: the differentiation of the subspecies *japonica* into two groups, a 'temperate' one comprising varieties of the temperate regions and high-altitude tropical regions and a 'tropical' one including the upland rice varieties. This differentiation is based on only a few loci and matches the morphological differentiation of Oka (1958).

Finally, two additional RFLP probes characterize the cytoplasmic diversity as a function of earlier data that enabled the identification of two major types, at the chloroplast level (Dally and Second, 1990) as well as the mitochondrial level (Second and Wang, 1992). Figure 2b shows the distribution of two cytoplasmic types among 141 of the 147 varieties. It is clear that one of the types characterizes the subspecies *indica* and some intermediates, while the other type is found in all varietal groups.



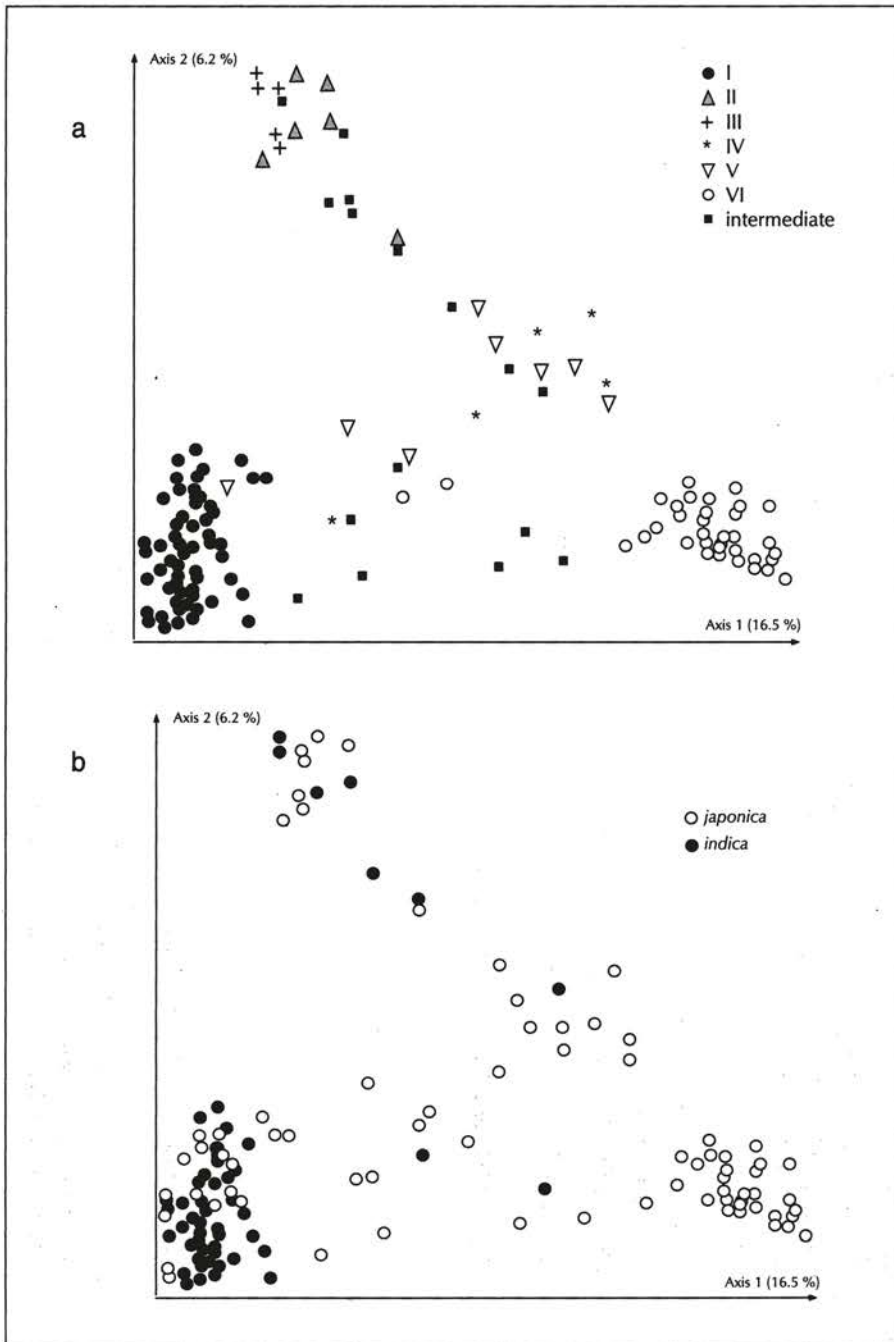


Fig. 2. Distribution on the 1-2 plane of the CA on RFLP data (202 nuclear probes and 482 bands) characterizing sample B, with (a) indication of enzymatic classification and (b) indication of cytoplasmic type (Dally and Second, 1990).

## MICROSATELLITES

The diversity of microsatellites was studied for 12 loci (*RM7*, *RM12*, *RM13*, *RM19*, *RM122*, *RM148*, *RM164*, *RM167*, *RM168*, *OSR4*, *OSR7*, *OSR35*) within a subgroup of 54 varieties representative of known elements of structure (see Appendix). The loci revealed 4 to 19 alleles per locus, with an average of 10.8. A CA was done on the 130 alleles obtained. The primary axes explained 6.0%, 5.8%, 4.8%, 4.7%, and 3.9% of the variation; this gradual decrease revealed a relatively weak structure. The 1-2 plane of the CA (Fig. 3) shows three groups: the first corresponds to group 1 of the enzymatic classification, the second to groups II and III, and the third to groups IV, V, and VI. Axes 3 and 4 separate the groups IV, V, and VI. On the other hand, groups II and III overlap. A great within-group diversity is observed.

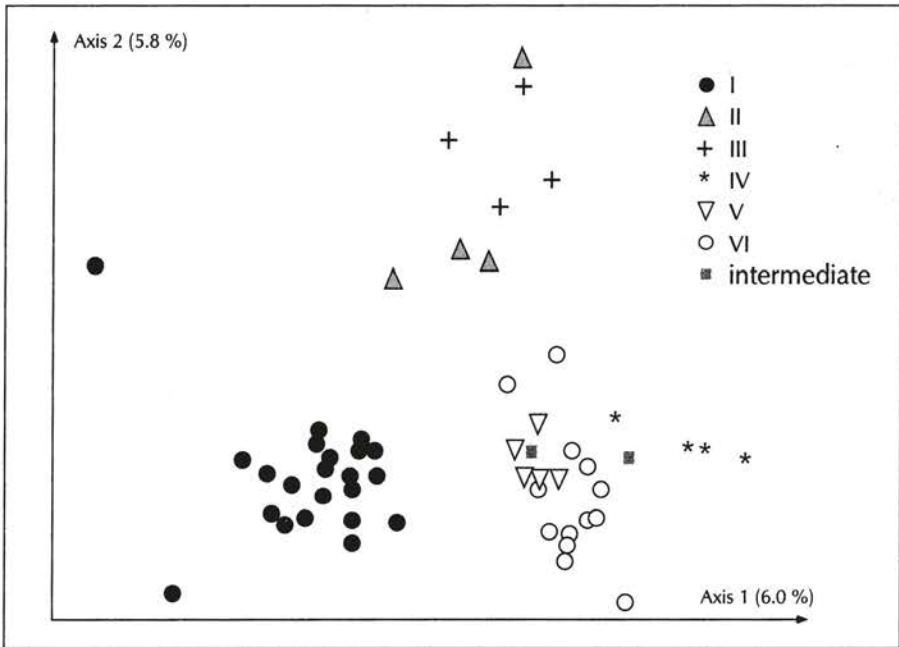


Fig. 3. Distribution on the 1-2 plane of the CA on microsatellite data (12 loci and 130 alleles) characterizing a sample of 54 varieties common to A and B.

## COMPARISON OF DIFFERENT MARKER TYPES

The broadest-based studies (isozymes) and the most intensive ones (RFLPs) give the same image of the species: a highly bipolar structure with nevertheless some peculiar, partly intermediate forms. With the microsatellites, the discrimination between the most differentiated groups is conserved, but a high polymorphism is revealed within the groups. Figure 4 shows the



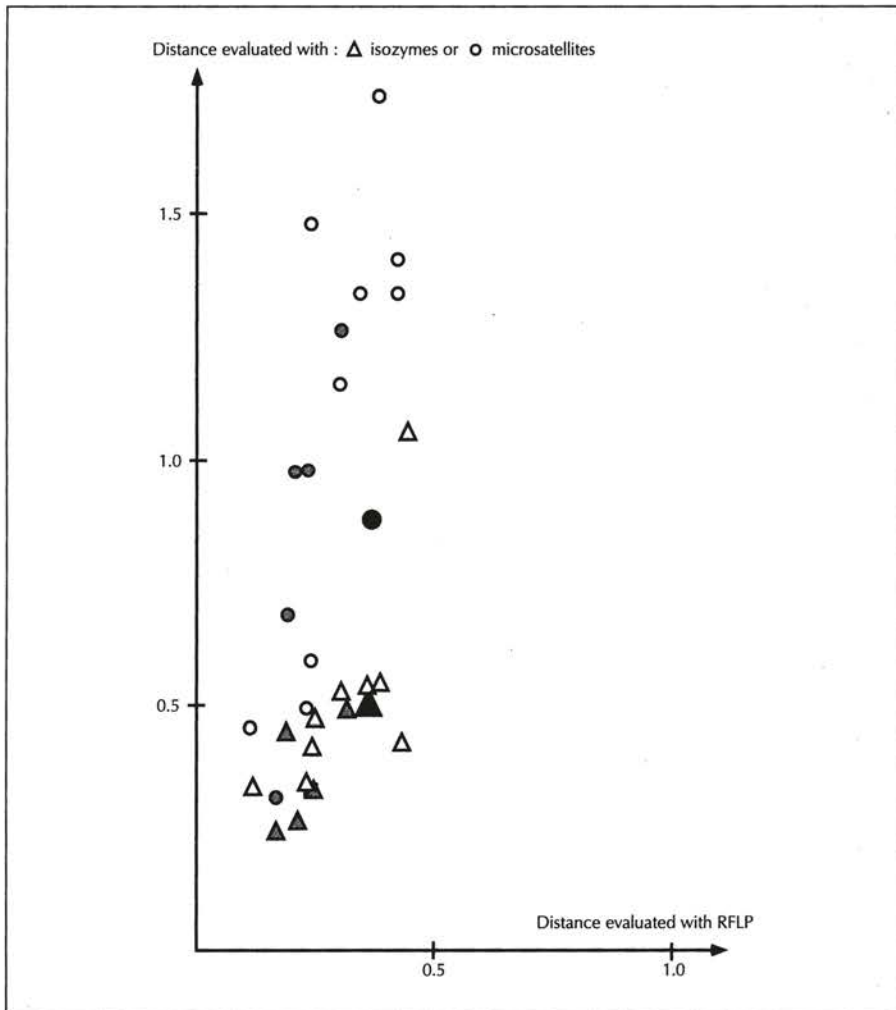


Fig. 4. Comparison between the distances evaluated with RFLP, isozymes, and microsatellites. The symbols in black characterize the distances between the two major groups I and VI; the symbols in grey characterize those between the large groups I, II, V, and VI; the symbols in white characterize those involving a small group, III or IV.

relationships between the intergroup genetic distances evaluated from different types of markers. The disparities between the numbers of accessions and loci taken into account for each of these types limit the conclusions that one can draw from this comparison. However, it is noted that, in comparison with RFLPs and isozymes, microsatellites considerably amplify the genetic distances between groups and that, among the comparisons between the largest groups (I, II, V, VI), the distance between groups I and VI is the largest with RFLPs and with isozymes, while it is far from being so with the microsatellites.

Thus, microsatellites reveal a greater polymorphism, which refines the resolution in the groups that are generally the least polymorphic. This polymorphism preserves the general imprint of the structure of the species but blurs the relations between groups.

## Molecular Classification and Diversity for Characters of Agronomic Interest

### MORPHOLOGICAL DIVERSITY

Data characterizing sample A were recovered from IRRI. A total of 248 varieties presented complete data for 11 variables; among these varieties all the enzymatic groups were represented except group IV, which flowered with difficulty in IRRI conditions. These data were subjected to a principal components analysis (PCA). The percentage of variation explained by the axes was 31.3%, 19.1%, 12.0%, 7.9%, and 7.0% for axes 1 to 5. Figure 5 shows the distribution of accessions in the 1-2 plane. Axis 1 separates the varieties with long organs (ligules, leaves, stems, and, to a lesser extent, panicles) and a rather thin grain, which are found close to negative values, from

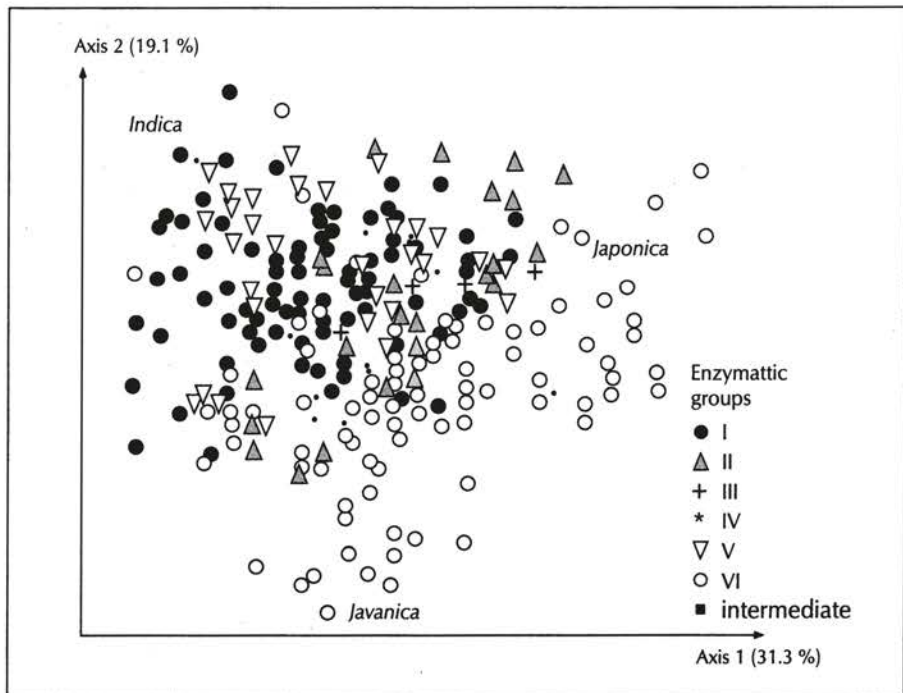


Fig. 5. Distribution in the 1-2 plane of the PCA of the variation among 248 varieties for 11 morphological characters

complementary varieties, which are on the side of positive values. Axis 2 draws towards the negative values the varieties with low tillering, large leaves, rather thick stem, and a wide and heavy grain. Thus, a tripolar structure is recognized with Indica types in the upper left corner of the plane, Japonica types in the extreme right corner of the plane, and Javanica types in the lowest part of the plane.

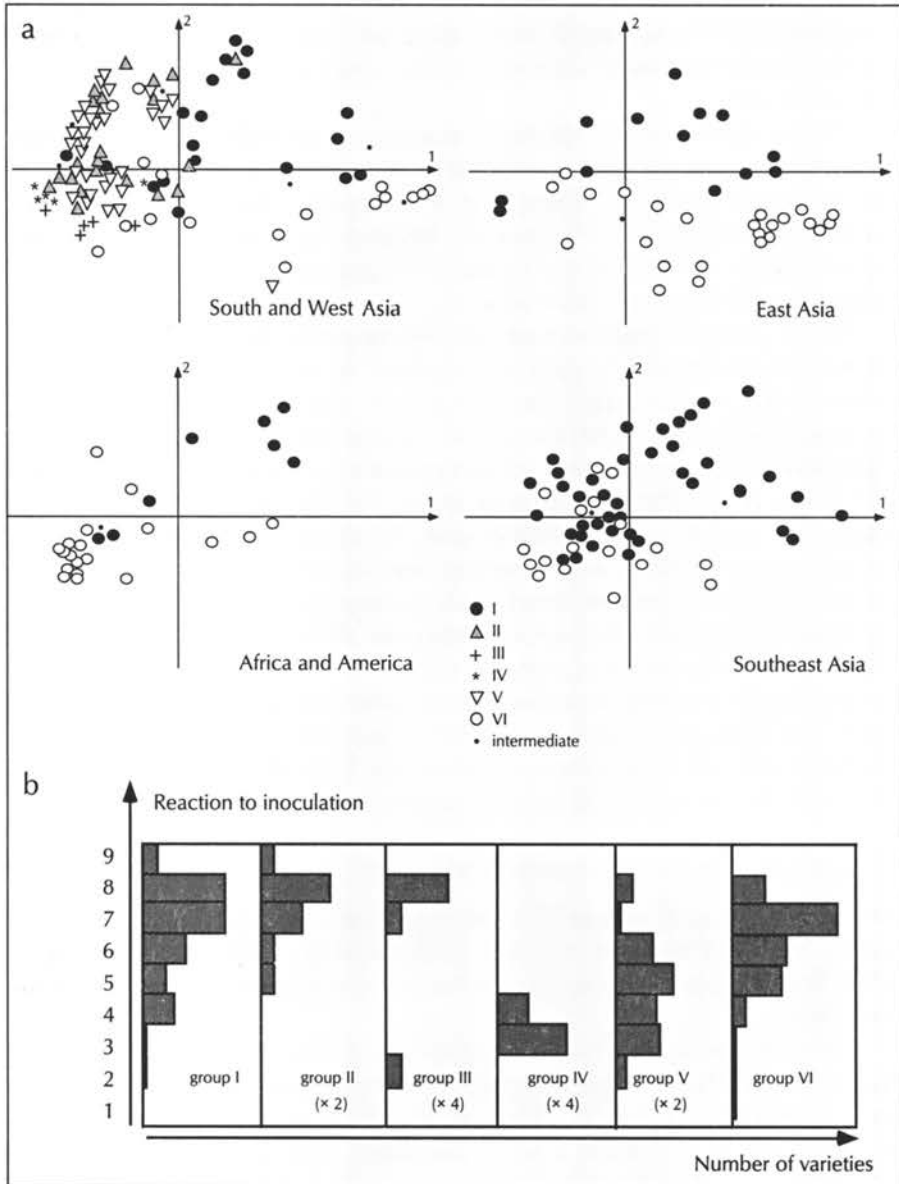
The morphological type Indica covers highly diversified groups in terms of molecular markers, with mostly representatives of groups I and V, but also of groups II and VI. The typical morphological types of Japonica and Javanica correspond only to group VI. While groups I and VI occupy nearly separate areas of the plane, the accessions of groups II to V are more frequent at the edge of these two major zones.

Within group VI, highly dispersed over the plane, there is a differentiation between the geographic origins that opposes the temperate forms (Japonica group) to the tropical forms (Javanica group). Between these two extremes, varieties of temperate, subtropical, and tropical origin are found, but mostly high-elevation tropical varieties from mountainous regions of Southeast Asia and South Asia. This distribution illustrates the observations made by Glaszmann and Arrauadeau (1986): with the morphological types Japonica and Javanica, two forms were observed that were differentiated on a narrow genetic basis and represent the extremes of a continuous variation that follows a geographic cline according to the latitude and altitude. The classic distinction between these two types is probably due to the fact that the first studies of morphological diversity were done on samples in which the original types of Japan and Java, areas more accessible to collectors than the mountains of Southeast or South Asia, were overrepresented. The RFLP data show a similar cline with the dispersion of varieties on axis 3 of the CA.

#### DIVERSITY OF REACTION TO VARIOUS PATHOGENS

The 270 varieties of sample A were tested for their reaction to the major fungal, bacterial, and viral diseases (Bonman et al., 1990; Glaszmann et al., 1995). This sample revealed a great diversity of responses. Two examples are given here.

Blast, caused by the fungus *Magnaporthe grisea*, is the principal fungal disease of rice. There is a great variability in the fungus and several resistance genes are known in the plant. The location of these resistance genes is a major task. Accessions of sample A were inoculated with 13 fungal strains in a collaborative study. The symptoms were recorded on the basis of the size of the lesions detected on several plants for each accession, according to a scale of 0 to 5. The average score for an accession was used to characterize its resistance to the strain tested. A PCA was done on the complete data set obtained for 257 accessions (Fig. 6a). Axis 1 separates the varieties that are generally resistant (negative values) from the varieties that are generally



**Fig. 6.** Reaction of varieties representing different enzymatic groups with respect to several diseases. (a) Blast resistance of varieties representing different enzymatic groups: distribution of 257 varieties on the 1-2 plane of the PCA on the size of lesions produced by inoculation of 13 strains of *Magnaporthe grisea*. (b) Relative tolerance of 6 enzymatic groups to tungro virus: distribution of reactions to inoculation by vector (scored 1 for complete absence of symptoms to 9 for maximum susceptibility) on a set of 261 varieties representing different enzymatic groups.

susceptible. In South Asia, where all the enzymatic groups are present, groups II to V are differentiated from major groups I and VI by a higher level of resistance. Among the varieties most often susceptible, axis 2 tends to differentiate the reference *indica* varieties (group I) from the reference *japonica* varieties (group VI), because of the susceptibility of each group to several differential strains. Within group VI, a differentiation is found between the temperate forms (South and East Asia) and the tropical forms (Southeast Asia, Africa, America). It is not possible to say whether these differences are due to a general differentiation that marks the ancient isolation between these forms or to a coevolution of host and pathogen. The clearest fact to be retained pertains to the diversity of responses obtained from varieties of neighbouring geographic origin, which are *a priori* exposed to pathogen populations that are not differentiated or only weakly differentiated, as a function of their belonging to different molecular groups. Thus, the groups of the molecular classification present marked differences in behaviour, with differences in frequencies of resistance, and high specialization of the reference strains.

Tungro is a viral disease of primary importance. To evaluate the resistance of each genotype, plants were inoculated by a vector and the reactions were recorded on a scale from 1 for complete absence of symptoms to 9 for maximal susceptibility. Figure 6b shows the distribution of reactions on a set of 261 varieties according to their molecular groups. Tolerance is clearly more frequent in groups IV and V.

A pioneering study showed that blast strains are classified into two groups as a function of their differential reaction against *indica* and *japonica* varieties (Morishima, 1969). A later study on bacterial blight showed that certain genes of complete resistance are distributed largely according to the groups of the enzymatic classification (Busto et al., 1990). Our results confirm these observations and allow us to conclude that the structuration of rice into varietal groups is essential to an understanding of the host-pathogen interactions and to finding new sources of resistance.

## Association between Characters and the Dynamics of the Genome

### DIVERSITY AT MADAGASCAR, INSULAR SITE OF EVOLUTION

Madagascar represents a peculiar case. Asian rice was introduced there in ancient times. The fact that it is an island made it an evolutionary laboratory for cultivated rice by limiting the influences, apart from a few major introductions of, most likely, Indian, Malaysian, or Indonesian origin. The observation by breeders of varietal types particular to Madagascar justified a detailed analysis of the diversity found on the island (Rabary et al., 1989; Ahmadi et al., 1991).

A collection of Madagascar varieties was studied from morphological and isozymic perspectives. Some peculiar morphological types intermediate between the Indica and Javanica types were found with high frequency (Fig. 7). The enzymatic diversity revealed was high, but much lower than that of the cultivated rices of Asia—the H index of diversity of Nei was 0.29 in Madagascar against 0.62 in Asia. The genotype structure is clearly bipolar and recalls the *indica-japonica* opposition. However, some particular types carry an allele coding for an aminopeptidase,  $Amp_1^2$ , which is very rarely observed in enzymatic groups I and VI in Asia. Among these types, some classic associations between isozymes and morphological characters are weak, even nonexistent.

These peculiarities suggest that several recombinations between varietal groups occurred since rice was first introduced in Madagascar. Despite these recombinations, the associations between the isozyme markers of the *indica-japonica* differentiation were maintained, while they involved genes located on several chromosomes. This situation demonstrates that it is possible to recombine genes involved in control of plant architecture, but it also indicates selection pressures, natural and human, that tend to maintain the gametic disequilibrium implied in the *indica-japonica* differentiation. Numerous enzymatic markers seem located in zones of the genome subject to these

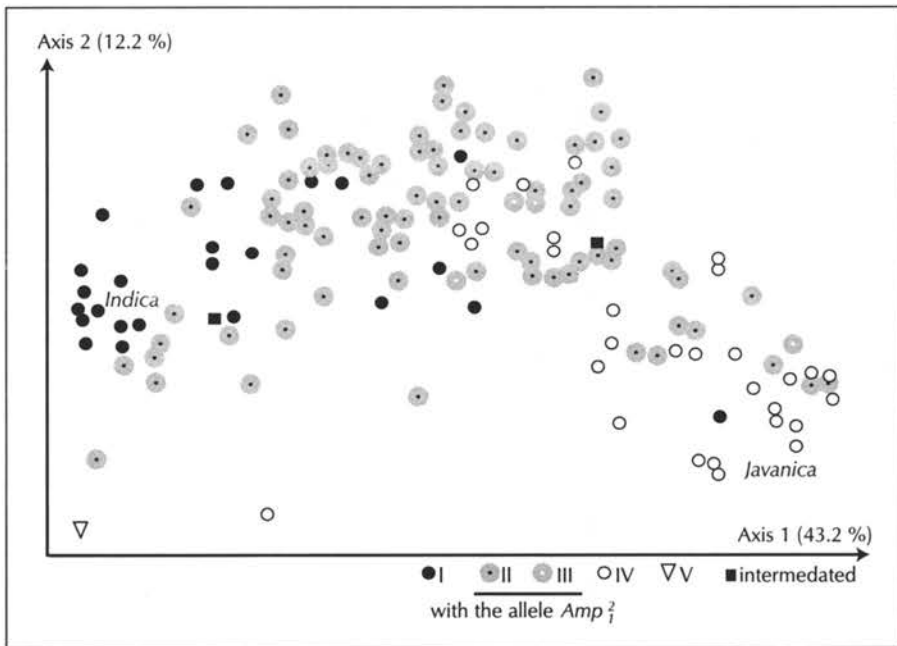


Fig. 7. Distribution of 144 varieties on the 1-2 plane of the CA on 39 morphological characters in the Madagascar material. The same symbols as in the preceding figures indicate the affiliation to enzymatic groups; a supplementary distinction is made for varieties possessing the allele  $Amp_1^2$ .



pressures, which confirms the value of classification based on enzymatic analysis.

#### THE CYTOPLASMIC GENOME AND ORIENTATION OF GENE FLOWS

From the particular situation of Madagascar, a phenomenon probably generalized in Asia has been documented, that of introgression between *indica* and *japonica*, source of diversity of the cultivated rices (Second, 1982).

Second and Ghesquiere (1995) compared nuclear and cytoplasmic characteristics within sample B. Two cytoplasmic types were distinguished, which was consistent with the global interpretation of the analysis of the genus *Oryza* for molecular markers: an '*indica*' type, I, and a '*japonica*' type, J (Dally and Second, 1990; Second and Wang, 1992). The *japonica* cytoplasmic type is found only in varieties of group VI, but together with the *indica* type (distribution J:I around 3:1) in the varieties of intermediate groups II to V. It is also present with a significant frequency among the varieties of group I (distribution J:I around 1:3). The *japonica* cytoplasm would thus have been introgressed by a number of varieties of group I, which indicates, given the maternal heredity of cytoplasm, that the hybrids at the origin of the introgressions of nuclear genes between the *indica* and *japonica* subspecies were all formed by hybridization of a female *japonica* parent pollinated by an *indica*.

#### DISTRIBUTION OF MOLECULAR DIVERSITY ALONG THE GENOME

The distribution of polymorphism along the genome presents a bias in contrast with what could be a random distribution (Second and Ghesquiere, 1995; Zhuang et al., 1998). It has been observed that monomorphic loci have a significant tendency to group in the species. Similarly, there is a tendency for *indica* markers to cluster together as *japonica* markers do (Second et al., 1995). This indicates clearly that the chromosomes of rice could be interpreted as a mosaic of regions that arise either from the ancestral type *indica* or from the ancestral type *japonica* and of monomorphic regions, which genetic drift could have fixed within the species in its present state from either one or the other of the ancestral types. The case of chromosome 12 is a good example of this phenomenon (Albar, 1998). There are strong associations, verified on several enzyme-probe combinations, between alleles of the same origin, *indica* or *japonica* (Fig. 8). This linkage disequilibrium is observed between loci that are very close (called a), located in the centromeric zone of chromosome 12, or between these loci and loci that are clearly further apart (called b). In this framework, monomorphic or nearly monomorphic loci and the loci for which the polymorphism is independent of the *indica-japonica* structure (called c, d, and e) could be intercalated. These loci represent probably areas subject to selection pressures of a different kind, as has been observed around certain isozymic loci in the Madagascar varieties.

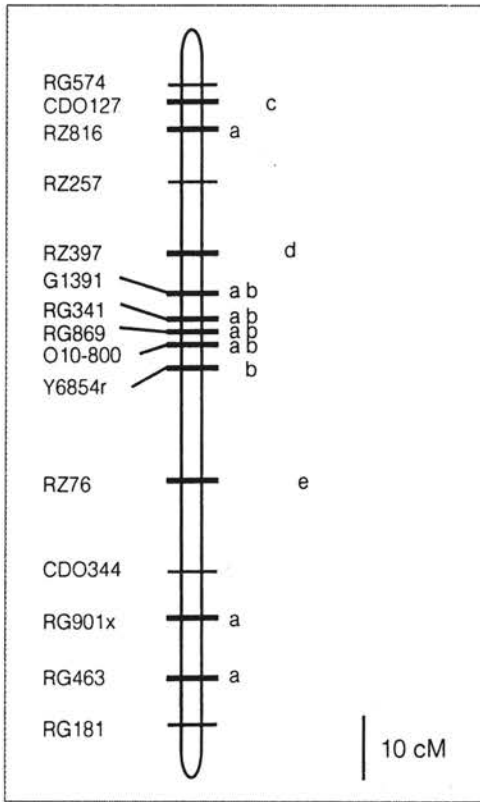


Fig. 8. Linkage disequilibrium along chromosome 12. On the basis of analysis of polymorphism within sample B, 11 loci out of the 15 tested were found to be polymorphic and are indicated by a thick horizontal bar. Linkage disequilibrium between loci was tested on the basis of the two most frequent alleles. Loci with significant disequilibrium ( $p < 0.01$ ) between them are indicated by the presence of a single letter next to the horizontal bar.

## CONCLUSION

Cultivated rices from Asia display a very wide diversity. The *indica* and *japonica* groups represent almost two different species if one accepts the hypothesis of a double origin from wild populations that were already differentiated (Second, 1982, 1984). Thus, there is a very marked structure, which is found whatever the type of marker used. The complementary inputs of markers such as isozymes and RFLPs on the one hand and microsatellites on the other are clearly seen. The isozymes and RFLPs give similar images and comparable indexes of differentiation. The RFLPs, because they are more numerous, allow an additional element to be seen: the ecogeographic differentiation between the temperate and tropical Japonica forms. Microsatellites less effectively record an ancient structure (*indica-japonica*), but they reveal an extended variability within each component.

Molecular markers bring to light important elements that are inaccessible through the study of morphological diversity alone: several groups (I to V) are identified within a group considered homogeneous in morphological terms. On the other hand, a clear continuity appears between two different

morphological types, Japonica and Javanica, which corroborates the conclusions drawn from the behaviour of progenies resulting from hybridization between the two types: they are generally fertile and are easy to fix through successive selfing generations.

As compared to earlier works on the species structure, molecular markers draw attention to particular forms, represented by groups II to V of the enzymatic classification. These results confirm various observations made by breeders, such as the particular behaviour of the 'aus' types (group II) in crosses with typical *indica* and *japonica* varieties (Wan and Ikehashi, 1997) or that of 'basmati' varieties (group V), of *Indica* morphology but very difficult to exploit in combination with high-yielding varieties of group I (G.S. Khush, personal communication). Our results on the behaviour of varieties exposed to the major diseases also illustrate the specificities of these groups, which can help to broaden the sources of resistance present in the major groups. These groups were identified through an approach of structured sampling. A preliminary set of 1688 Asian varieties was put together by diversifying the geographic origin and ecosystem as much as possible and taking local classifications into account (Glaszmann, 1987). This sample was subsequently extended to other continents. The structuration into enzymatic groups was used to overrepresent the subgroups rich in particular diversity. This process has helped to constitute a 'nucleus' collection of a manageable size for refined evaluations.

The results of these evaluations confirm the general view. It has been observed, for example, that the minor groups harbour original sources of resistance to blast or tungro and that the japonica varieties of Africa are generally more resistant to blast than those of Asia. These associations within the sample indicate simple parameters—geographic origin, type of cultivation, classification established using a few selected markers—that it is advisable to diversify in order to enrich the collection. On the contrary, one could use the results to quickly find varieties that are exceptions to the associations or to the general correlations in order to move towards a precise selection. For example, to produce varieties with few tillers and long, thick panicle (general characteristics of group VI), but also fine grain (characteristic of other groups), one could select varieties that have the finest grain among the varieties of group VI, which generally have bold grains. The process would be more effective than if these characters were recombined between the groups. The complications linked to intergroup recombinations, all of which indicate that they disturb the association of coadapted factors, would be avoided. Then one would benefit from the accumulation of factors of grain fineness in certain varieties that have the desired genetic background, whether this accumulation be the result of a juxtaposition of factors dispersed in the populations of group VI or the result of the successful introgression of factors specific to other groups.

Our observations indicate a multitude of associations between molecular and quantitative characters, because of the existence of varietal groups differentiated for the two types of characters, as observed by Virk et al. (1996). Such associations have schematically two principal determinants.

The first is a foundation effect at the time of domestication, which possibly reinforces the joint action of natural selection and genetic drift that have differentiated the founding populations. The probably separate origin of the *indica* and *japonica* types derived from forms that diverged 2 to 3 million years ago (Second, 1985) lends a particular importance to this phenomenon in Asian rice and explains the omnipresence of associations. The origin of minor groups remains to be established. It is nevertheless clear in the light of our results that, even in the absence of an overall satisfying interpretation, the molecular data can constitute a valuable source of information to diversify the sources of useful genes. This has already proved of practical use (Kameswara-Rao and Jackson, 1997).

The second determinant is the introgression between ancestral types. Several studies, including ours on rice from Madagascar, show that gene exchanges between varieties of different types are frequent. Nevertheless, if there was a generalized recombination, the associations would disappear rapidly over time. The possibility of evaluating the molecular diversity with markers mapped on the genome is of great interest in this context. The results available suggest that molecular polymorphism is distributed on the genome in non-random segments (Second and Ghesquiere, 1995; Zhuang et al., 1998). It could have a discrimination power differentiated, particularly for the varietal group II, according to the position of these markers in relation to the centromeres (Parsons et al., 1997). These phenomena merit more detailed study, as has been the case for chromosome 12 (Albar, 1998). In the search for the QTL for partial resistance to rice yellow mosaic virus, the indication of interactions between markers—or between markers and QTL—dispersed on the genome but belonging each time to zones that have a high *indica-japonica* linkage disequilibrium may illustrate the importance of phenomena of coadaptation in a crop such as cultivated rice of Asian origin (Pressoir et al., 1998). In the present state of our understanding, we hold that the apparent associations are the result of two phenomena: linkage disequilibrium that involves tightly linked genes and markers and associations between coadapted genes that could be dispersed on the genome.

The establishment of a database of molecular data and detailed evaluation for agronomic characters on pure lines that are results of controlled crosses as well as on unrelated lines will allow us to accumulate results and better understand and use the dynamics of evolution of genetic diversity of rice.

## APPENDIX

### Plant Material

The results presented are an overall perspective on earlier data that have already been partly published and new data.

On the basis of information compiled before 1985, a preliminary sample of 270 varieties, sample A, was put together to represent the geographic, ecotypic, and enzymatic diversity of cultivated rices of Asian origin. The samples were purified—through one plant selection and controlled selfings—to serve as a core collection to study the diversity for useful characters (Bonman et al., 1990; Glaszmann et al., 1995). These varieties are here used to compare the classification obtained from isozymes with the morphological diversity and the diversity of response to several diseases. The morphological data are extracted from the database maintained by the IRRI. The data on reactions to various pathogens are those reported by Glaszmann et al. (1995).

A second sample of 147 accessions, sample B, served in analysing the average diversity of RFLP for 202 mapped nuclear probes covering the entire rice genome. Among these accessions, 141 were analysed using two probes of cytoplasmic DNA: a probe located at the mutation point 28 of chloroplastic DNA (Dally and Second, 1990) and the mitochondrial probe Col (Second and Wang, 1992).

The subgroup common to A and B, which comprises 54 varieties, was used to study the diversity revealed by microsatellites: 12 loci were taken into account.

Another sample of 144 Madagascar varieties was studied to compare the molecular and morphological classifications in an island environment isolated from the area of origin of rice (Ahmadi et al., 1991). Twenty-four quantitative characters coded according to a distribution into large classes as well as 15 qualitative characters were used.

### Data Analysis

A principal component analysis was done on quantitative data and correspondence analysis on qualitative data. Various marker types were compared by quantifying the genetic diversity between varietal groups using the Nei index of distance (1978).

## REFERENCES

- Ahmadi, N., Glaszmann, J.C., and Rabary, E. 1991. Traditional highland rices originating from intersubspecific recombination in Madagascar. In: *Rice Genetics II*. Manila, Philippines, IRRI, pp. 67-79.
- Albar, L. 1989. Etude des bases génétiques de la résistance partielle du riz au virus de la panachure jaune (RYMV): cartographie moléculaire et relation avec la diversité génétique. Doct. thesis, Université Paris XI, Paris, 187 p.
- Blair, N.W., Panaud, O., and McCouch, S.R. 1999. Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, 98: 780-792.
- Bonman, J.M., Mackill, A.O. and Glaszmann, J.C. 1990. Resistance to *Gerlachia oryzae* in rice. *Plant Disease*, 74: 306-309.
- Busto, G.A., Ogawa, T., Endo, N., Tabien, R.E., and Ikeda, R. 1990. Distribution of genes for resistance to bacterial blight of rice in Asian countries. *Rice Genetics Newsletter*, 7: 127-128.
- Chang, T.T. 1976. The origin, evolution, cultivation, dissemination, and diversification of Asian and African rices. *Euphytica*, 25: 435-441.
- Chang, T.T., Adair, C.R., and Johnston, T.H. 1984. The conservation and use of rice genetic resources. *Advances in Agronomy*, 35: 37-90.
- Cheng, K.S., Wang, X.K., Zhou, J.W., Lu, Y.X., Lou, J., and Huang, H.W. 1984. Studies on indigenous rices in Yunnan and their utilization. 2. A revised classification of Asian cultivated rice. *Acta Agronomica Sinica*, 10: 271-280 (in Chinese).
- Dally, A.M. and Second, G. 1990. Chloroplast DNA diversity in wild and cultivated species of rice (genus *Oryza*, section *Oryza*): cladistic-mutation and genetic-distance analysis. *Theoretical and Applied Genetics*, 80: 209-222.
- Glaszmann, J.C. 1985. A varietal classification of Asian cultivated rice (*Oryza sativa* L.) based on isozyme polymorphism. In: *Rice Genetics I*. Manila, Philippines, IRRI, pp. 83-90.
- Glaszmann, J.C. 1987. Isozymes and classification of Asian rice varieties. *Theoretical and Applied Genetics*, 74: 21-30.
- Glaszmann, J.C. 1988. Geographic pattern of variation among Asian native cultivars (*Oryza sativa* L.) based on fifteen isozyme loci. *Genome*, 30: 782-792.
- Glaszmann, J.C. and Arraudeau, M. 1986. Rice plant type variation: Japonica-Javanica relationships. *Rice Genetics Newsletter*, 3: 41-43.
- Glaszmann, J.C., Benoit, H., and Arnaud, M. 1984. Classification des riz cultivés (*Oryza sativa* L.): utilisation de la variabilité isoenzymatique. *L'Agronomie tropicale*, 39: 51-66.



- Glaszmann, J.C., Mew, T., Hibino, H., Kim, C.K., Vergel de Dios-Mew, T.I., Vera Cruz, C.H., Notteghem, J.L., and Bonman, J.M. 1995. Molecular variation as a diverse source of disease resistance in cultivated rice. In: *Rice Genetics III*. Manila, Philippines, IRRI, pp. 460-465.
- Ishii, T., Brar, D.S., Second, G., Tsunewaki, K., and Khush, G.S. 1995. Nuclear differentiation in Asian cultivated rice as revealed by RFLP analysis. *Japanese Journal of Genetics*, 70: 643-652.
- Jackson, M.T. 1997. Conservation of rice genetic resources: the role of the international rice genebank at IRRI. *Plant Molecular Biology*, 35: 61-67.
- Kameswaro-Rao, N. and Jackson, M.T. 1997. Variation of seed longevity of rice cultivars belonging to different isozyme groups. *Genetic Research and Crop Evolution*, 44: 159-164.
- Lolo, O.M. and Second, G. 1988. Peculiar genetic characteristics of *Oryza rufipogon*. *Rice Genetics Newsletter*, 5: 67-70.
- Mackill, D.J. 1995. Classifying japonica rice cultivars with RAPD markers. *Crop Science*, 35: 889-894.
- Morishima, H. 1969. Differentiation of pathogenic races of *Pyricularia oryzae* into two groups, indica and japonica. *SABRAO Newsletter*, 1: 81-94.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89: 583-590.
- Oka, H.I. 1958. Intravarietal variation and classification of cultivated rice. *Indian Journal of Genetics and Plant Breeding*, 18: 78-89.
- Parsons, J.B., Newbury, H.J., Jackson, M.T., and Ford-Lloyd, B.V. 1997. Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. *Molecular Breeding*, 3: 115-125.
- Pressoir, G., Albar, L., Ahmadi, N., Rimbault, I., Lorieux, M., Fargette, D., and Ghesquiere, A. 1998. Genetic basis and mapping of the resistance to rice yellow mottle virus. 2. Evidence of a complementary epistasis between two QTLs. *Theoretical and Applied Genetics*, 97: 1155-1161.
- Rabary, E., Noyer, J.L., Benyayer, P., Arnaud, M., and Glaszmann, J.C. 1989. Variabilité génétique du riz (*Oryza sativa* L.) à Madagascar: origine de types nouveaux. *L'Agronomie tropicale*, 44: 305-312.
- Second, G. 1982. Origin of the genetic diversity of cultivated rice (*Oryza sativa* L.): study of the polymorphism scored at 40 isozyme loci. *Japanese Journal of Genetics*, 57: 25-57.
- Second, G. 1985. Evolutionary relationships in the *Sativa* group of *Oryza* based on isozyme data. *Génétique, sélection, évolution*, 17: 89-114.
- Second, G. and Ghesquiere, A. 1995. Cartographie des introgressions réciproques entre les sous-espèces indica et japonica de riz cultivé (*Oryza sativa* L.) In: Colloque techniques et utilisations des marqueurs moléculaires. Paris, INRA, les Colloques de l'INRA no. 72, pp. 83-93.

- Second, G., Parco, A., and Caiole, A. 1995. The hybrid origin hypothesis of cultivated rice (*Oryza sativa*) explains some of the gaps in its RFLP maps and suggests an efficient mapping population for useful genes and QTLs. In: *Plant Genome and Plastome: Their Structure and Evolution*. K. Tsunewaki, ed., Tokyo, Japan, Kodansha Scientific, pp. 129-136.
- Second, G. and Wang, Z.Y. 1992. Mitochondrial DNA RFLP in genus *Oryza* and cultivated rice. *Genetic Research and Crop Evolution*, 39: 125-140.
- Virk, P.S., Ford-Lloyd, B.V., Jackson, M.T., and Newbury, H.J. 1995. Use of RAPD for the study of diversity within plant germplasm collections. *Heredity*, 74: 170-179.
- Virk, P.S., Ford-Lloyd, B.V., Jackson, M.T., Pooni, H., Clemeno, T.P., and Newbury, H.J. 1996. Predicting quantitative traits in rice using molecular markers and diverse germplasm. *Heredity*, 76: 296-304.
- Wan, J. and Ikehashi, H. 1997. Identification of two types of differentiation in cultivated rice (*Oryza sativa* L.) detected by polymorphism of isozymes and hybrid sterility. *Euphytica*, 94: 151-161.
- Wang, Z.Y. and Tanksley, S.D. 1989. Restriction fragment length polymorphism in *Oryza sativa* L. *Genome*, 32: 1113-1118.
- Yang, G.P., Sanghai Maroof, M.A., Xu, C.G. Zhang, Q., and Biyashev, R.M. 1994. Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. *Molecular and General Genetics*, 245: 187-194.
- Zhang, Q., Sanghai Maroof, M.A., Lu, T.Y. and Shen, B.Z. 1992. Genetic diversity and differentiation of *indica* and *japonica* rice detected by RFLP analysis. *Theoretical and Applied Genetics*, 83: 495-499.
- Zhou, H., Glaszmann, J.C., Cheng, K.S., and Shi, X. 1988. A comparison of methods in classification of cultivated rice. *Chinese Journal of Rice Science*, 2: 1-7 (in Chinese).
- Zhu, J., Gale, M.D., Quarrie, S., Jackson, M.T., and Bryan, G.J. 1998. AFLP markers for the study of rice biodiversity. *Theoretical and Applied Genetics*, 96: 602-611.
- Zhuang, J.Y., Qian, H.R., and Zheng, K.L. 1998. Screening of highly-polymorphic RFLP probes in *Oryza sativa* L. *Journal of Genetics and Breeding*, 52: 39-48.