Sugarcane

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Sugarcane is a major product of the tropical and subtropical zones. The cultivation of sugarcane is the basis of the sugar industry. At present, world production of sugar is more than 100 million tonnes a year, around 70% from cane and 30% from beet. The largest producers are India, Brazil, Cuba, and China.

Cultivated cane has a classic morphology of perennial grass. It is propagated by stem cuttings from which axillary buds develop. Sugar continues to accumulate in the stem past the vegetative period, even after flowering has taken place. It is triggered by the combined action of relative cold and a drop in the water supply (Fauconnier, 1991). In equatorial climates, where there is no marked dry season, there is often a low level of sugar in the stems.

The cycle between two harvests varies from 10 to 24 months depending on the climate and economic considerations. The cycle between two plantations is highly variable and depends mostly on socioeconomic criteria. For example, it overlapped with the harvest cycle in Hawaii and may extend over more than 10 regrowths in certain unfavourable areas in Reunion. The growth is initiated directly from cuttings called 'virgin' cuttings. The yield is generally maximal at the first growth and then tends to decrease with every harvest.

TAXONOMY AND GENETIC RESOURCES

Biology, Taxonomy, and Geographic Distribution

Cane is a monocotyledon of the family Poaceae. The inflorescence is slack and ramified, and flowers in panicles are arranged in pairs, one sessile, the other pedunculate. Each flower is bisexual and has three stamens and an ovule. All the stems do not necessarily flower. The flowering intensity depends on genetic and climatic factors. Like most grasses, the plant is wind-pollinated. Flowering is generally considered unfavourable to production and is thus

selected against. The fruit is a caryopse. It is used only for the purpose of selection and never as seed in cultivated fields.

The genus *Saccharum* belongs to the tribe Andropogonea, as do two major cereals, maize and sorghum. It originated from Asia (Fig. 1). Taxonomists distinguish five basic species.

Saccharum officinarum, the first species cultivated, probably originated from Papua New Guinea. Clones of this species have think stems that are very rich in sugar.

Saccharum barberi originated in India and *S. sinense* in China. Clones of these two species are generally more hardy than those of *S. officinarum*. They have stems that are finer, more fibrous, and less rich in sugar. They result from spontaneous hybridizations between *S. officinarum* and the wild species *S. spontaneum* (D'Hont et al., 2002).

Saccharum spontaneum is a wild species with a vast geographic distribution, which covers nearly all of Asia, Afghanistan, and the Pacific islands. The different ecotypes may be annual or perennial. They have high morphological variability.

Saccharum robustum, another wild species, is probably the ancestor of *S. officinarum*. It is found essentially in Papua New Guinea, where it forms dense populations along the rivers.

All the species of the genus *Saccharum* are polyploid. The clones of *S. officinarum* have 80 chromosomes. This number was established on the basis of certain observations, and it is possible that the few clones that do not have 80 chromosomes are in fact hybrids with other species (Bremer, 1924). The clones of *S. barberi* and *S. sinense* have chromosome number varying from 81 to 124. Most are probably aneuploids. For *S. spontaneum*, the chromosome number varies from 40 to 128 depending on the clones, and at least 21 different cytotypes have been observed in India. A large number of clones are aneuploid, but clones that have a chromosome number that is a multiple of 8 are most frequent. For *S. robustum*, there are two major cytotypes: $2n = 60$ and $2n = 80$ (Sreenivasan et al., 1987). The recent studies of *in situ* hybridization on chromosomes (fluorescent *in situ* hybridization or FISH) prove that the base number x is 8 for *S. spontaneum* and 10 for *S. officinarum* and *S. robustum* (D'Hont et al., 1998).

In all the species of the genus *Saccharum*, the chromosomes at meiosis paired mainly in bivalents (Price, 1963). Irregularities such as the formation of univalents or multivalents are nevertheless frequently observed (Burner, 1991; Burner and Legendre, 1993).

The Evolution of Cultivated Forms

Domestication and diffusion of clones

Most researchers agree that the domestication of *S. officinarum* occurred in Papua New Guinea and in the neighbouring islands (Fig. 1). First, there is an

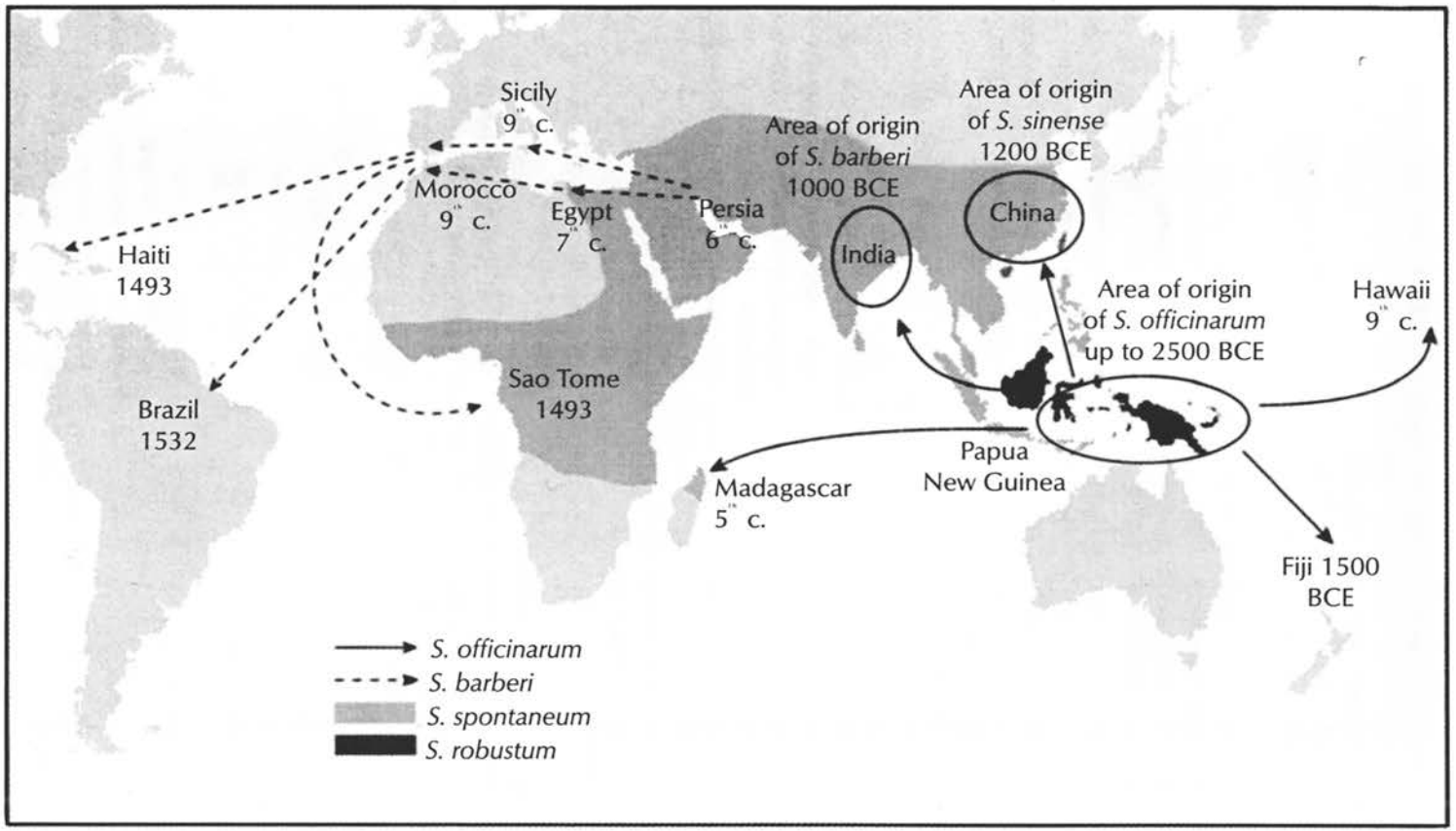

Fig. 1. Area of origin of the three domesticated species of sugarcane, *S. officinarum*, *S. barberi*, and *S. sinense*, and dispersion up to the beginning of the 16th century (Blume, 1985; Daniels and Roach, 1987; Meyer, 1989).

exceptional morphological diversity of clones of *S. officinarum* in this region. Second, the local wild species *S. robustum*, which was the origin of *S. officinarum*, is present there (Daniels and Roach, 1987). The domestication of cane dates from about 2500 BCE. Cane was cultivated to be chewed and there was probably no development of a sugar extraction industry in its area of origin.

Austronesian migrations spread the cultivation of *S. officinarum* eastward, to the South Pacific islands, and towards the northwest, to India and China, around 1500 to 1000 years BCE. The species *S. barberi* and *S. sinense* appeared at this stage, in India and China respectively. These two countries were probably the centres of origin of the sugar extraction industry.

Cultivation spread outside the area of origin in several major stages. *Saccharum officinarum* was probably disseminated with human migrations as a chewed cane, from Melanesia towards a large part of the tropical islands of the Pacific during the first millennium. The Europeans discovered this species only in the 18th century during the first exploratory voyages in the Pacific. *Saccharum barberi* spread from India towards the west, as a raw material of the sugar industry. In the year 500, Persia was a reputed site of sugar production. The Arabs extended its cultivation to North Africa and to the Mediterranean islands. The Portuguese and then the Spanish subsequently introduced it in the 15th century to the islands in the Atlantic (Madeira, Canary Islands, Cape Verde, Sao Tome). Finally, this species crossed the Atlantic during the second voyage of Christopher Columbus and was acclimatized for the first time in America on the island of Haiti. During the two centuries that followed, the extension of cane cultivation in the Americas, mainly in Brazil and then in the Caribbean, was closely linked to European colonization and, as a corollary, to the plantation economy and the slave trade.

Propagation of cane cultivation from India towards the west during the first millennium, its introduction in America, and then the development of plantations up to the middle of the 18th century were all accomplished from a single clone—or from a very small number of clones—called Creole in the Antilles. It was probably a clone of *S. barberi* or a hybrid between *S. barberi* and *S. officinarum*.

During the middle of the 18th century, European explorers brought clones of *S. officinarum* from the South Pacific. Their cultivation developed rapidly in South and Central America. These clones, because they were rich in sugar, were called 'noble' canes. Their use was quickly developed in the plantations. The clone Bourbon, also called Vellai, Otaheite, and Lahaina, occupied most of the cultivated area up to the middle of the 19th century and then, under parasitic pressure, it was replaced by other clones such as Lousier, the series of Cheribons, or Tanna (Stevenson, 1965).

Although collections in Southeast Asia and the South Pacific played a significant role in clonal renewal, natural mutants of cultivated varieties were

also successful to some extent. For example, Lousier was a mutant of Bourbon and the Cheribon series corresponded to a group of coloration mutants arising from a single clone.

Use of sexual reproduction

The inflorescence of cane was recognized as such and described in the 18th century but it was only in the mid-19th century, in the Barbados islands, that the seeds were observed for the first time (Stevenson, 1965). The first breeding programmes started simultaneously in Barbados and Java around 1890 and, at the beginning of the 20th century, there were already six breeding centres in the world. The breeders at first concentrated on crosses between noble clones of *S. officinarum* and reported some success. In Java, the clones POJ100 and EK28 resulted from programmes of intraspecific crossing. They allowed significant progress in sugar productivity on the islands.

The first work on interspecific hybridization started in Java with the installation of the breeding centre Proefstation Oost Java, in the beginning of the 20th century. It relied on 'nobilization', a term created by the Dutch to designate the process of crossing a noble clone of *S. officinarum*, rich in sugar, with a vigorous or disease-resistant clone of a related species, then backcrossing the hybrid on the noble species, possibly several times. The result is a cultivable phenotype that conserves the useful character contributed by the related clone.

At the time, Java plantations were ravaged by mosaic, a disease caused by a potyvirus, and by sereh, a disease probably of viral origin, which no longer exists (Rands and Abbott, 1964). Since no source of resistance was found in *S. officinarum*, breeders used Chunnee, a resistant clone of *S. barberi* imported from India. The progenies were no longer sensitive to sereh, but they had poor sugar yield and remained sensitive to mosaic. However, some descendants, such as POJ213, were cultivated on a large scale in other regions of the world and used with success as progenitors in several breeding centres, especially in India.

The utility of interspecific crosses was proved in the 1920s. At that time, breeders discovered in Java the clone Kassoer, which was probably a spontaneous hybrid between Black Cheribon, a cultivated clone of *S. officinarum*, and Glagah, a local clone of *S. spontaneum*. Kassoer was nobilized once by POJ100 and a second time by EK28. Among the progeny, the Dutch researchers selected POJ2878, an exceptional clone rich in sugar and resistant to mosaic and sereh. Just eight years after the original cross, POJ2878 occupied 90% of the cane industry of Java and subsequently spread throughout the world. This clone also had considerable success as progenitor in most of the breeding centres.

In India also, at the Coimbatore station, interspecific crosses were performed from the beginning of the 20th century. A commercial hybrid,

Co205, was obtained after a single generation of nobilization (F_1 hybrid) between Bourbon and a local clone of *S. spontaneum*. This success is a unique example of acquisition of a useful commercial phenotype after a simple crossing without backcrossing on a noble clone. The breeders subsequently developed trispecific hybrids by crossing their F_1 hybrids *S. officinarum-S. spontaneum* with the *S. officinarum-S. barberi* hybrids of the POJ213 type produced in Java. The best varieties in Coimbatore were produced in this way.

The first interspecific hybrids developed at the Proefstation Oost Java and at Coimbatore (POJ2878, Co290, etc.) are in the pedigree of almost all the varieties presently cultivated.

Despite these successes, the narrowness of the genetic base of commercial varieties remains a major concern for many breeders. Arceneaux (1967) studied the pedigree of 114 varieties developed in the major breeding centres during the period 1940-1964. He showed that the clones used to develop these varieties were of limited number: 19 clones of *S. officinarum*, of which 4 played a particularly important role (Black Cheribon, Bandjarmasin Hitam, Loethers, and Crystalina); some clones of *S. spontaneum*, especially a clone with 2n = 112 originating from Java (Glagah) involving a single gamete in interspecific hybrid Kassoer, and one or several clones with 2n = 64 originating from India, called local Coimbatore; a clone of *S. barberi* (Chunnee); and a clone of *S. robustum*, present only in the pedigree of some varieties produced in Hawaii. These numbers contrast with the hundreds of clones of various other species that have been studied and are conserved in different collections (Berding and Roach, 1987).

Faced with this situation, breeders took up the work of nobilization in several centres, in Australia, Barbados, and Louisiana, in the 1960s (Roach, 1978, 1986; Berding and Roach, 1987). More recently, clones belonging to the genera *Erianthus* and *Miscanthus* were used as a source of wild material. These attempts at intergeneric widening of the genetic bases have not yet given significant results.

The structure of the genome

The varieties resulting from nobilization have made possible an enormous increase in sugar yields. *Saccharum spontaneum* has certainly contributed factors of resistance to several diseases. The worldwide success of the first hybrid clones suggests that they have also acquired a better general adaptation to cultural conditions, with especially greater vigour and tillering and better resistance to drought and cold (Panje, 1972; Roach, 1986). At the genome level, the contribution of *S. spontaneum* has been determined by particular mechanisms of transmission. The first generations of interspecific crosses and backcrosses have seen the transmission of 2n chromosomes by the *S. officinarum* clone used as female parent, while the male parent would transmit

the normal gamete number n. The result is that the modern cultivars have a chromosome number between 100 and 130 depending on the clones with around 10% of these chromosomes derive from the wild species.

Using *in situ* hybridization (genomic *in situ* hybridization or GISH), we can now differentiate the chromosomes according to their parental origin (D'Hont et al., 1996). For example, studies on the variety R570 ($2n \cong 112$) show that nearly 10% of chromosomes come from the species *spontaneum* and 10% from recombinations between chromosomes of the two parental species. For the variety NCo376, there are around 112 chromosomes, of which nearly 25 are derived from *S. spontaneum* and 11 from interspecific recombinations.

The molecular mapping of RFLP (Grivet et al., 1996) indicates that the two ancestral species, which do not have the same basic chromosome number, are differentiated only by some simple chromosomal rearrangements. The pairing of chromosomes at meiosis seems to be mostly the polysomic type, a typical behaviour of autopolyploids. However, some preferential pairings have been observed between certain chromosomes resulting from the species *S. spontaneum*. That could explain the relatively limited number of recombinations between chromosomes of the two species.

Genetic Resources

Cane is propagated in the field by stem cuttings. The production of seeds is often possible, but highly destructuring in the genotypic sense in this highly polyploid and heterozygous plant. Material is exchanged essentially in the form of stem cuttings.

The scientific and interdisciplinary community is highly organized. The International Society of Sugar Cane Technologists (ISSCT) brings together most of the research institutions and researchers working on sugarcane. It works with national societies in each country. Apart from information exchange, the ISSCT ensures the coordination and sometimes financing of activities of general interest. In the field of genetic resources, the ISSCT participates in different activities such as the collection of material and publication of standards to be observed for material exchanges. There is thus an authority and a framework for the conservation and circulation of genetic resources, as well as a convention of exchanges between varietal improvement centres.

Collection operations rely on international cooperation between sugarcane research institutions, the ISSCT and IPGRI (International Plant Genetic Resources Institute), with the authorization of the countries involved.

The samples collected are deposited in two international collections: one in India (Cannanore and Coimbatore) and one in the United States (Miami and Canal Point). These collections presently contain about 2500 accessions each.

The interest and support of the community for the collection of genetic resources of sugarcane, however, has not been followed up by the requisite efforts towards their conservation, evaluation, and use. The main gene banks have reached a size that is not compatible with the maintenance and systematic evaluation of all the accessions. Thus, a large number of clones of the US collection have been lost: nearly 100% of the primary clones in the collection and close to 50% of the clones collected in 1976 and 1977, mainly because of diseases or natural disasters (Comstock et al., 1996).

The Indian collection is in much better condition (Roach, 1992; Alexander and Viswanathan, 1996). It comprises 3345 accessions, of which more than half are directly derived from collection expeditions. It is maintained in three regions with complementary environments: in Coimbatore by the Sugarcane Breeding Institute, for species resistant to mosaic, especially *Erianthus* and most of the *S. spontaneum* clones; in Cannanore by the same institute, for most of the other materials (except *Miscanthus*) because this region is free from mosaic; in Willington by the Indian Agricultural Research Institute, for the clones that cannot be conserved at low altitude, particularly the representatives of the genus *Miscanthus*. Very few clones conserved in these regions have been lost. Moreover, to compensate for accidental losses in the cultivation of field clones, an *in vitro* collection is being established. It is important to mention that the Indian collection receives no international financial support.

There are small collections in the producer countries, but they are working collections for breeders rather than collections to conserve genetic resources.

The international collections regularly contribute to breeding programmes partly based on the objective of widening the genetic base. Exchanges and transport of plant material in the form of cuttings carry the serious risk of transfer of pathogens that must be controlled. Very strict rules are adopted and sites located outside the cultivation regions have been identified to set up quarantine services.

The international community showed interest in the conservation and exchange of genetic resources during two workshops held in the 1990s: the ISSCT workshop, held at CIRAD in Montpellier in March 1994, on the theme of genetic resources of sugarcane, and an international workshop on the conservation and exchange of genetic material, organized by the Australian cane community, in Brisbane, in June 1995.

The first meeting was an occasion to formulate priorities for genetic resource management—especially in favour of inventories and more systematic information exchanges—and standardization of methods of description, particularly for molecular markers. The need for a core collection was affirmed. Such a collection could be constructed from a grouping and analysis of existing data, then diffused across the world for complementary characterization. The second meeting accounted more specifically for the phytosanitary constraints that limit plant material exchanges and represent

a permanent challenge to researchers and plant protection services (Croft, 1996).

Framework of Application of Molecular Markers

Analysis of the genetic diversity of sugarcane with molecular markers was taken up from the late 1960s with isozymes (Heinz, 1969) and flavonoids (Williams et al., 1974; Daniels and Daniels, 1975). These studies, as well as those that followed, contributed important information on the structure of the genus *Saccharum* and on its relationships with other genera (Daniels and Roach, 1987; Glaszmann et al., 1989; Eksomtramage et al., 1992). The markers based on DNA polymorphism began to be used at the end of the 1980s to study the diversity within the genus *Saccharum* (Glaszmann et al, 1990; Burnquist et al., 1992). Studies were conducted subsequently on more particular material, from a few cultivars to representatives of genera related to cane (Al-Janabi et al., 1994; Sobral et al., 1994; Harvey and Botha, 1996; Besse et al., 1997; Burner et al., 1997). CIRAD researchers, in collaboration with several partners, completed various studies linked to objectives of genetic improvement.

The objective of applying molecular markers was to better understand the evolutionary history that resulted in the cultivated forms and to find to what extent molecular diversity can have a predictive value for characters useful to breeders.

The diversity of agronomic value within the genus *Saccharum* has not been the subject of broad-based studies. This may be because of the very high plasticity of characters, which makes them difficult to evaluate. Genetic interpretations are limited, since they are strung together from sources of variation as different as the number of chromosomes, which ranges from single to triple in *S. spontaneum*, and occasional mutations such as those that accompanied the clonal evolution of cultivated varieties. Moreover, such studies in the basic species have a low impact because the morphological description of material used for an interspecific hybridization has nearly no predictive value for the progeny that results from it (Simmonds, 1993).

The most detailed analysis of the morphoagronomic variation in cultivars was carried out in Cannanore, in south India (Nair et al., 1998). It covered nearly 400 cultivars from 10 geographic origins and addressed essentially the quantitative characters involved in sugar yield. There is a clear indication that cultivars from different origins do not show the same adaptation to the test site. Schematically, the two primary factors of the multivariate analysis express a highly variable level of performance in this environment, one being constructed from correlations between various measures of cane production (stem height, cane weight, cane yield), the other from correlations between various measures of saccharose content. The third factor is determined by the conventional opposition between stem size and diameter. This part of

the agromorphological variability of modern cultivars is probably essentially determined by their interspecific hybrid origin and the equilibrium between the various genomic components originating from *S. officinarum* and *S. spontaneum*.

The application of molecular markers has had several specific objectives. First of all, the phylogenic hypotheses formulated to explain the relations between species of *Saccharum* had to be tested. Then the nuclear diversity revealed by RFLP within the material presently cultivated had to be analysed. Since this material was derived from interspecific hybridizations involving *S. officinarum* and *S. spontaneum*, the results were examined in reference to the diversity of these two species. In a third phase, researchers sought to determine whether the diversity has conserved traces of what is the principal characteristic of the origin of modern cultivars of sugarcane: interspecific hybridization from a very limited number of accessions followed by only a few generations of intercrosses from the first interspecific products. The expected consequence was the existence of linkage disequilibrium associating certain markers; the result hoped for was the possibility of extending this reasoning to genes of agronomic interest and to target future molecular studies so that they refine the comprehension of genetic bases of the diversity useful for breeders.

ORGANIZATION OF MOLECULAR DIVERSITY

Relationships between *Saccharum* Species and the Cultivars

Before considering the molecular diversity among the cultivars, it is useful to situate them among the major species of the genus.

Cytoplasmic diversity

The cytoplasmic diversity was studied by D'Hont et al. (1993) using heterologous chloroplast and mitochondrial probes to reveal the RFLP among 58 clones representing different groups of the *Saccharum* complex, as well as some cultivars. The chloroplast probe used, even though it covers nearly 20% of the chloroplast genome in wheat, enabled differentiation only of the genera *Saccharum*, *Erianthus*, and *Miscanthus*. The eight mitochondrial probes used allowed differentiation of 10 types of profile. Among the 18 *S. spontaneum* clones, a wide variability was revealed, with the existence of six types of profile following a distribution of 11, 2, 2, 1, 1, 1. No clear relation appeared between this diversity and the geographic origin of clones. The 15 *S. robustum* clones showed two profiles, which were distinguished by a single band following the distribution 13, 2. The clones of three cultivated species, *S. officinarum*, *S. barberi*, and *S. sinense*, showed a single profile, identical to the dominant profile of *S. robustum*. The diversity of the mitochondrial genome

is in accordance with the taxonomic relationship between the wild species. The results are compatible with the hypothesis that *S. officinarum* arose from *S. robustum*. They also agree with a hybrid origin for *S. barberi* and *S. sinense* by introgression between *S. officinarum* and *S. spontaneum*, *S. officinarum* being the female parent. The few cultivars studied showed the same profile as clones of *S. officinarum*.

Nuclear diversity

The nuclear diversity was studied by Lu et al. (1994a, b) using simple copy nuclear probes on the basis of a collection of 51 clones representing different species of *Saccharum* and 39 cultivars. The hybridization profiles for each clone showed a large number of bands with variable intensities, which reflects the complex polyploid structure of species. Most of the probe-enzyme combinations revealed 10 to 60 bands among the clones of the collection and 10 to 40 bands among the cultivars. In total, 1106 polymorphic bands were read from 36 probe-enzyme combinations. Most of the bands were present in only a few clones since 61% were found in fewer than five genotypes and 25% in only one genotype. This revealed a wide variability within the collection, particularly in wild species. In contrast, the common bands were more frequent among the cultivars, which reflected a close similarity between the cultivated genotypes.

A matrix was formed from 90 individuals and 1106 bands including some incomplete data, and different correspondence analyses (CA) were done with certain individuals or certain probe-enzyme combinations considered inactive to obtain complete matrixes. These analyses revealed similar overall images, whether they were based on the use of 5, 10, 20, or 30 probe-enzyme combinations. Figure 2 shows the overall distribution of genotypes obtained with 13 probe-enzyme combinations for which the data were complete for almost all the material. The three basic species, *S. spontaneum*, *S. officinarum*, and *S. robustum*, are clearly differentiated. The distribution according to axis 1 separates the clones of *S. spontaneum* from those of *S. robustum* and *S. officinarum*. *Saccharum robustum* and *S. officinarum* can be distinguished according to axis 2. The widest diversity is observed among the genotypes of *S. spontaneum* and then within the sample of *S. robustum*. The representatives of the two species *S. barberi* and *S. sinense* are distributed between *S. officinarum* and *S. spontaneum* in the proximity of the genotypes *S. officinarum*. These results are in agreement with the hypothesis that *S. officinarum* was introduced in India and China and pollinated by local forms of *S. spontaneum* to produce *S. barberi* in India and *S. sinense* in China. The cultivars are distributed between the clones of *S. officinarum* and *S. spontaneum* but are closer to the former. This reflects their interspecific origin, and also the nobilization scheme they have been subjected to, in order to acquire the principal characteristics of noble canes.

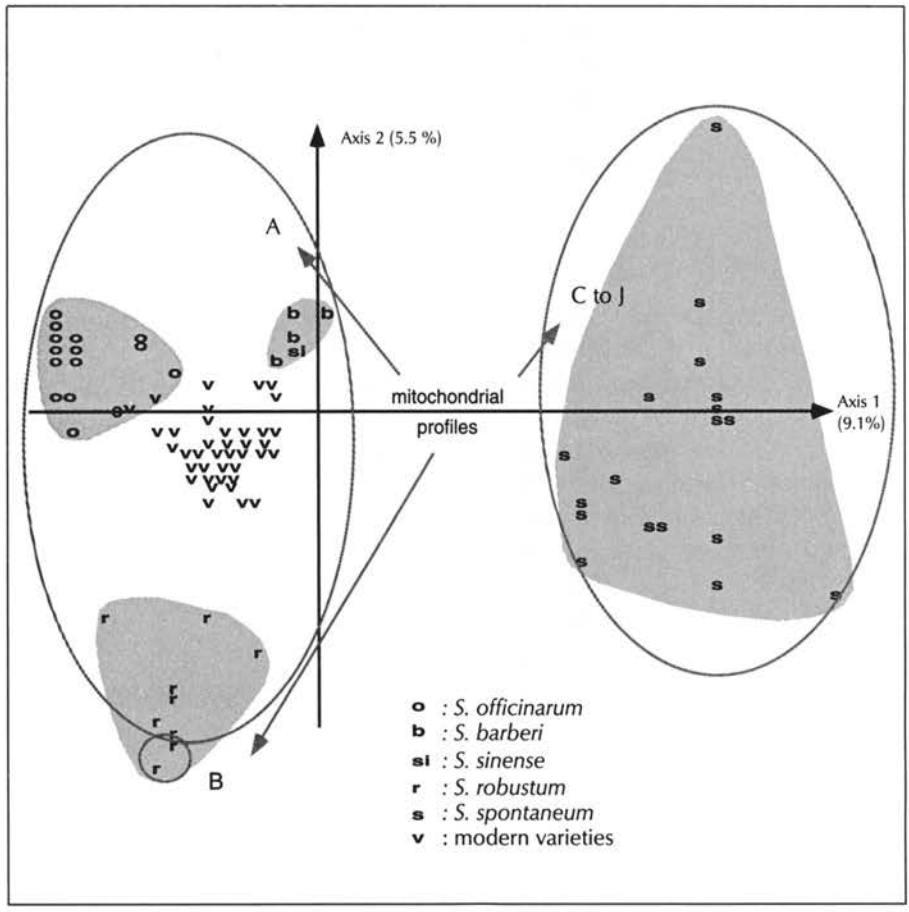

Fig. 2. Distribution of 89 wild or cultivated sugarcane clones in the 1-2 plane of a CA among 463 polymorphic RFLP bands obtained with 13 probes corresponding to single nuclear sequences. The distribution of various cytotypes listed using 8 mitochondrial probes is indicated. o, *S. officinarum*; b, *S. barberi*; si, *S. sinense*; r, *S. robustum*; s, *S. spontaneum*; v, modern varieties.

Diversity among the Cultivars

The diversity within the cultivars is influenced by the diversity within the ancestral species and by the transmission of that diversity during interspecific crosses, as well as by more refined factors of structuration linked to the intervention of breeders and the organization of the genome.

Diversity within the ancestral species

The studies of Lu et al. (1994a) revealed considerable diversity within *S. spontaneum*. The probability that a band present in a clone will also be present in another clone that has been compared to it (Dice index) is 0.31. However,

a significant structuration can be observed: multivariant analysis of the data extracted for *S. spontaneum* allowed differentiation of the genotypes of India with a low chromosomal number from the Southeast Asia and East Asia genotypes. This is in agreement with the cytogeographic classification of Panje and Babu (1960), which distinguishes the genotypes of the central region (India and Afghanistan) from those of the eastern region (China, Southeast Asia). The great diversity observed among the genotypes of the eastern group suggests the possibility of a finer subdivision.

The diversity within *S. officinarum* was studied by Lu et al. (1994a) and then more precisely by Jannoo et al. (1999b). In the latter study, a sample of 53 *S. officinarum* clones were analysed by RFLP using 11 nuclear probes. The clones represented four particular subgroups: those from New Guinea, which is considered the centre of origin of the species; those from various Indonesian islands (Molucca, Celebes, Borneo); clones from several Pacific islands (Fiji, New Caledonia); and clones of uncertain origin that are widely implicated in the constitution of present cultivars. The nuclear probes were distributed throughout all the known linkage groups with the sugarcane genome and were used in combination with one or two restriction enzymes. A total of 305 bands were detected. Out of 53 clones analysed, two pairs of completely identical clones were detected and 51 unique profiles were conserved for the subsequent steps of the analysis. The clones present an average of 4.5 to 7.5 bands per profile. This large number of bands characterizes the high level of ploidy of the species and high general heterozygosity. It is apparent from analysis of the distribution of this parameter that there is a subgroup of nine clones that present a higher heterozygosity than the others.

Figure 3 shows the distribution of clones on the 1-2 plane of a CA based on these data. Two cases are distinguished. If all the clones are considered (Fig. 3a), the structure is essentially determined by the genotypes that have the largest number of bands: 7 genotypes grouped in the right part of the plane and 2 genotypes in the extreme position in the upper part. These clones represent particular forms of the species, which present a concentration of infrequent alleles. Their greater heterozygosity (higher number of bands) may indicate a hybrid origin with other compartments of the genus or of the complex *Saccharum*. If the clones that have the highest number of bands are excluded in order to limit the analysis to a more homogeneous *S. officinarum* compartment, the distribution of clones becomes more continuous (Fig. 3b). Some peculiar forms are found, originating especially from New Caledonia, related to one of the genotypes located at an extreme position in Fig. 3a and removed from this last analysis. The New Guinea clones are distributed throughout the lower part of the plane, with, however, a high concentration in the centre. The clones of the Indonesian islands and those involved in the genealogy of cultivars have a distribution close to that of the New Guinea clones. The Fiji clones are intermediate between the New Caledonia forms and the remaining clones.

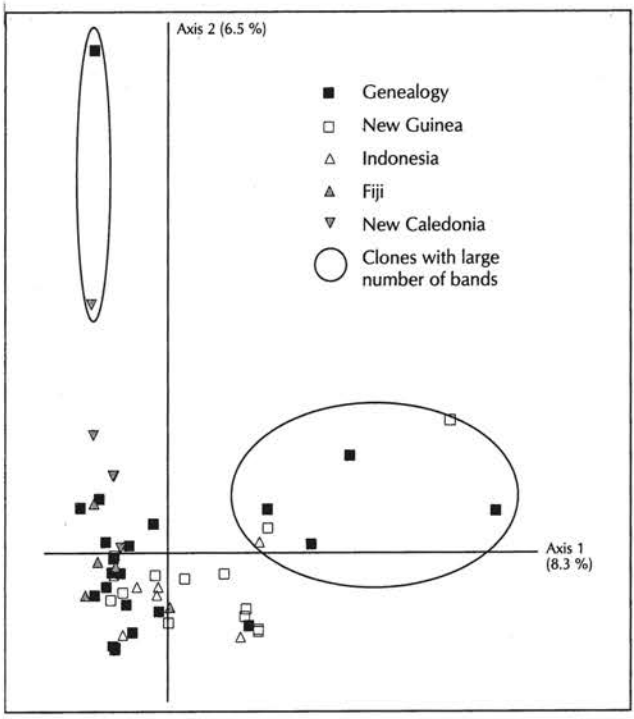

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Fig. 3a. Distribution of 51 genotypes of *S. officinarum* in the 1-2 plane of a CA among 305 polymorphic RFLP bands obtained with 11 probes corresponding to unique nuclear sequences.

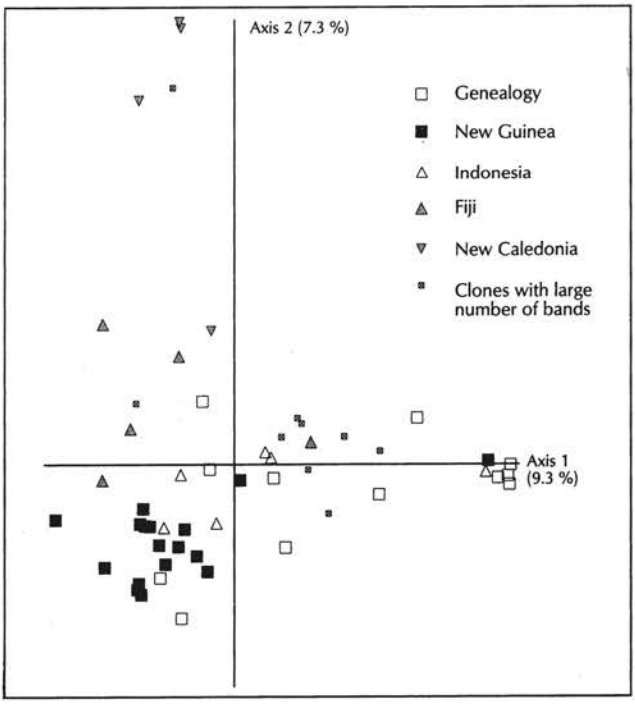

Fig. 3b. Distribution of 42 genotypes of *S. officinarum* in the 1-2 plane of a CA among 252 polymorphic RFLP bands obtained with 11 probes corresponding to unique nuclear sequences. Nine clones having a large number of bands are also projected.

A general structuration probably determined by introgressions from other species or genera can thus be observed in *S. officinarum*. Among the forms that seem to be free from such an influence, the variation is significant, since we find a high heterozygosity, but it seems unstructured. The genotypes used to create the modern cultivars occupy a large part of the distribution of the species and seem to ensure good representation of the diversity of *S. officinarum* within the genome of cultivars.

Breaking down the diversity in cultivars

Molecular diversity within the cultivars has been studied by Lu et al. (1994b) on the basis of a sample of 39 varieties of diverse origin, then by Jannoo et al. (1999b) from 109 cultivars mainly resulting from breeding programmes in Barbados and Mauritius. This latter study involved 11 probes combined with one or two restriction enzymes and detected 336 polymorphic bands. The variability within the cultivars was characterized first of all by a larger number of bands than in *S. officinarum*: 7.4 against 5.5 bands per probe-enzyme combination. The CA revealed several important elements.

The variability of the *S. officinarum* clones involved in the genealogy of cultivars is represented without apparent bias (Fig. 4a). A more detailed analysis of band frequencies shows that the majority of markers are found in the cultivars.

The structuring part of the diversity in cultivars is essentially contributed by *S. spontaneum* (Fig. 4b). The markers that contribute the most to the principal axes of the CA are generally absent from the group of *S. officinarum* clones.

The origin of cultivars may constitute a significant component of variability, even though it relies on some markers only. The gap between the Barbados clones and those of Mauritius is shown on the first axis of the CA (Fig. 4b), even though it may be linked to a difference of band frequency for 13 markers only. From the results we cannot determine the factors responsible for this differentiation, particularly whether they belong to the breeders' practices, such as the recurrent use of certain progenitors, or whether they denote an effect of differential adaptation to contrasting environments.

Fine structuration of polymorphism

The linkage disequilibrium was researched on 59 clones cultivated on the island of Mauritius or used as breeding material (Jannoo et al., 1999a). By restricting the analysis to material derived from a single breeding programme, we limit the factors of variation associated with geographic origin. Thus, the detection of associations between markers imputed to the single physical linkage on the chromosomes is favoured.

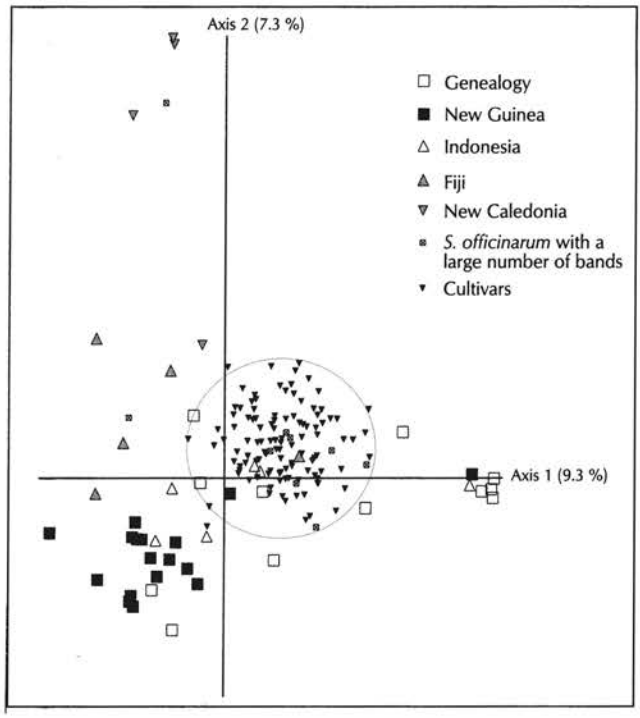

Fig. 4a. Distribution of 42 genotypes of *S. officinarum* in the 1-2 plane of a CA among 252 polymorphic RFLP bands obtained with 11 probes corresponding to unique nuclear sequences. In addition, 109 clones are projected.

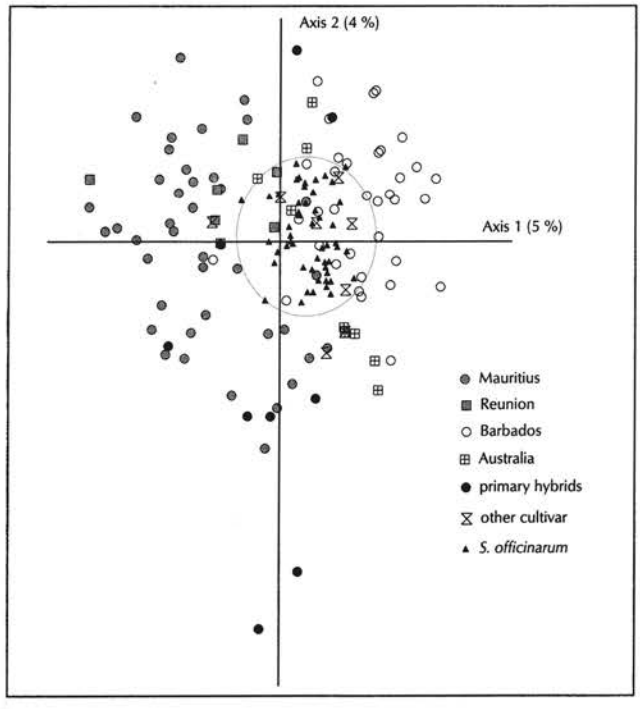

Fig. 4b. Distribution of 109 sugarcane cultivars in the 1-2 plane of a CA among 336 polymorphic RFLP bands obtained with 11 probes corresponding to unique nuclear sequences. In addition, 51 clones are projected.

What emerges from this analysis is that the association generally involves loci separated by less than 10 centimorgans (Fig. 5). Forty-two cases of association between at least two related loci are listed, representing allelic multilocus formulae present in at least one of the primary progenitors and thus probably transmitted by it at the beginning. Around two thirds of associations involve markers that seem to arise from *S. spontaneum*.

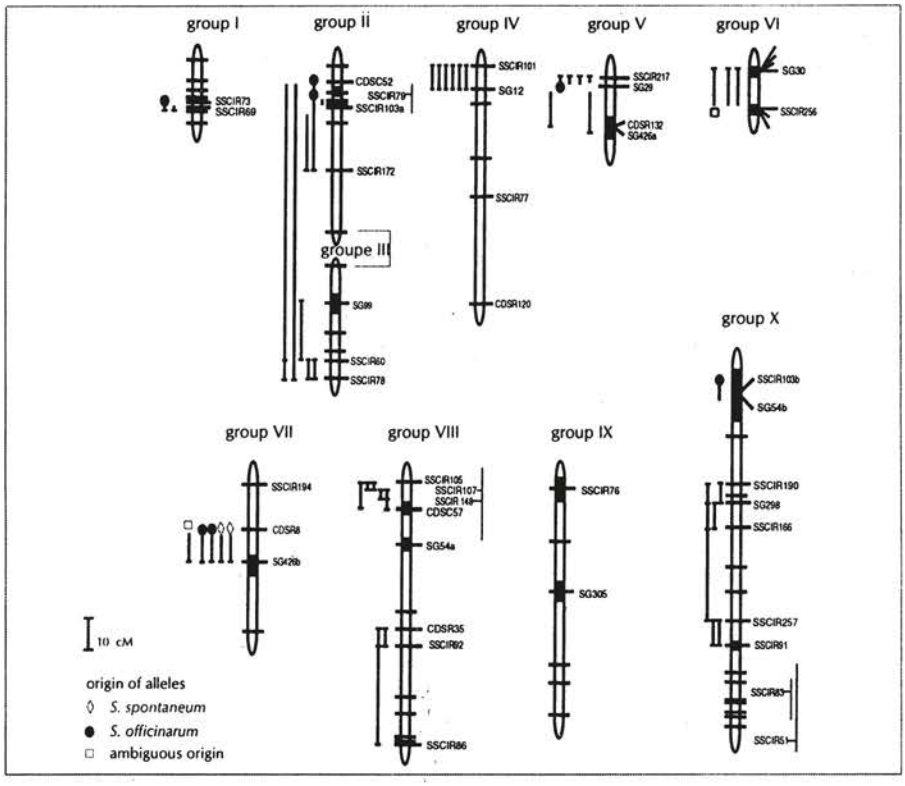

Fig. 5. Distribution of linkage disequilibrium detected among 59 cultivars along the composite map of cultivar R570. The 38 probes correspond to 41 loci indicated on the map. Loci with imprecise position are identified by a T bar on the right when they are isolated, and by a black bar when in a group. The loci involved in a linkage disequilibrium are indicated by a bar at the left of the linkage group. Several bars between the loci indicate that several preferential allelic associations are observed.

This component of the structuration of diversity within cultivars is interpreted as the result of a foundation effect (associated with the bottleneck) occurring when only a few interspecific hybrids are used. The associations thus created can be maintained through successive crosses when the physical linkage is strong enough.

CONCLUSION

The molecular markers used in these studies are RFLP markers. They allow a very detailed characterization of the material. In all the samples studied, more than half of the bands revealed were polymorphic. Because of the polyploid nature of the material and very high heterozygosity, each probe reveals generally more than 5 bands per individual. In taking into account a few probes, one rapidly gets the number of bands compared beyond the threshold of discrimination between the individuals. For example, Lu et al. (1994b) proposed a group of 5 probes that, combined with a single restriction enzyme, allowed the identification of all the cultivated varieties without ambiguity. For the same reasons, it is apparent that just a few probes are sufficient to give access to the general structure of the interspecific diversity.

The results confirm the previous phylogenetic hypotheses and bring complementary elements at the intraspecific scale. Within *S. spontaneum*, a geographic cline is observed in which the meridional forms with higher chromosome number are opposed to the septentrional forms. However, the variation at the cytoplasmic level presents a specific profile independent of the geographic origin. The variation in *S. officinarum* reveals an extended centre of diversity without tight structure and allows the detection of a secondary diversity, perhaps associated with introgressions with other compartments of the complex.

These results indicate that molecular markers, particularly RFLP, will be very useful in constituting a core collection of genetic resources of sugarcane. The nature of the international mechanisms of conservation and management of these resources justifies a laboratory investment close to the Indian collection, presently the richest and best-managed collection.

On the other hand, RFLP markers prove ineffective in examining a higher taxonomic level, particularly one that allows us to elucidate the events, allo- or auto-polyploidization, for example, that led to the polyploid *Saccharum* complex. Other, more global, markers such as GISH may be more useful.

Among the modern cultivars, RFLP markers allow us to first evaluate the genetic basis that is exploited in relation to the available resources, to analyse its structure, and to relate it to different components and various phenomena. Despite the low number of progenitors effectively used during interspecific crosses, considerable diversity is observed among the cultivated varieties today: polyploidy has ensured the maintenance of a wide genetic base. The linkage disequilibrium between closely related markers opens up perspectives for evaluation of the qualitative contribution of the primary progenitors in terms of genes of agronomic interest. The process of genome mapping and marking of these genes in the model progenies will allow us to follow their transmission in the material selected and to relate the molecular diversity of selected loci to the diversity of characters useful in selection.

APPENDIX

RFLP Analysis

The data produced contribute to the identification of polymorphic RFLP bands within the sample studied and to the construction of matrixes coded 0-1, corresponding to the absence or presence of these bands in the accessions analysed. Since clones are involved, each accession is represented by a single individual. The Dice index was calculated to quantify the similarity between two accessions, which corresponds to the percentage of common bands in relation to the number of bands present in at least one of two accessions being compared. The matrixes have been treated by CA. These CA have been done with all the data, then after certain markers or certain individuals were withdrawn from the active elements in the analysis. The too rare or too frequent markers may give an excessive weight to certain individuals and thus mask the overall structure. In all the cases of analysis presented here, markers showing a frequency less than 5% or higher than 95% were designated as supplementary. The possibility of designating certain individuals as supplementary allows us to withdraw very peculiar clones or to locate a given group of clones within a frame of reference based on the diversity of clones maintained as active. We have used this possibility to examine the respective contributions of ancestral species to the structuration of the diversity of cultivars.

Research on Linkage Disequilibrium

Thirty-eight probes mapped into 41 loci distributed throughout the genome were used, which enabled the detection of 1057 polymorphic bands. An exact Fisher test was done on all the data to compare the frequencies of association between the loci according to whether they belong or do not belong to the same linkage group. The same test was then applied in limiting the comparisons to markers of the same linkage group, to test whether strong linkages more frequently associate with bilocus allelic associations.

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