Rapport de mission à Cuba du 20 au 27 juin 1997

Journées scientifiques de l'INICA à l'occasion du cinquantenaire de la recherche sur la Canne à sucre à Cuba

Centre de coopération internationale en recherche agronomique pour le développement

Département des cultures annuelles CIRAD-CA Centre de Guadeloupe

Philippe Feldmann

Projet Connaissance du génome et amélioration variétale de la Canne à sucre

Octobre 1997

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Programme de la mission

Vendredi 20/06/97 : Pointe à Pitre –La Habana (CU 407)

Samedi 21/06/97 : La Habana (Dr. Cabrera et Bernal)

Dimanche 22/06/97 : La Habana – Varado (INICA)

Lundi et Mardi 23-24/06/97 : Journées scientifiques de l'INICA à Jovellanos (EPICA)

Mercredi 25/06/97 : Varado - Villa Clara
Laboratoire des sols et laboratoire de vitroplants
Retour sur La Habana

Jeudi 26/06/97 : CNIC, journée avec les chercheurs de Bioplantas

Vendredi 27/06/97 : La Habana – Pointe à Pitre

Introduction

A l'occasion du cinquantième anniversaire de la station de recherches EPICA de Jovellanos et du quarante-cinquième de celui de la station de recherches EPICA de Mayari, l'INICA a organisé un colloque de cinq jours sur trois localisations à Cuba.

L'INICA est la structure nationale fédérative qui regroupe les 13 stations de recherches locales (EPICA) des différents états de Cuba ainsi que la station de quarantaine située sur l' Isla de Juventud. Plus de 1700 personnes y travaillent.

La culture de la canne à Cuba occupe actuellement 1,5 millions d'hectares avec une production 1997 de 4,2 millions de tonnes de sucre. Cette production est très réduite par rapport à la période soviétique où Cuba disposait d'un marché protégé. Les problèmes économiques et politiques (blocus américain) limitent la remontée en production .

Les journées « anniversaires » de la Canne à sucre à Cuba

Le colloque a accueilli 150 participants venant principalement de Cuba et d'Amérique latine hispanophone (Guatemala, Costa Rica, El Salvador, Colombie, Equateur) avec une très forte participation mexicaine mais aussi des participants de Barbade (WISBEN : T. Kennedy), du Brésil (Copersucar : W. Burnquist), d'Espagne et d'Italie (Institut Agronomique de l'Outre-mer : F. Turchi).

De nombreuses communications et posters ont été présentés sur les trois sites du congrès (Jovellanos, Santa Clara, Mayari). Ils ont été édités sous forme de résumés en anglais et en espagnol et sous forme de disquette informatique[1].

Deux communications ont été présentées par le projet *Connaissance du génome et Amélioration variétale de la Canne à sucre* :

Feldmann, P., Rott, P., Oriol, P., Paulet, F., Daugrois, J., and Sapotille, J. (1997). Use of tissue culture of sugarcane for rapid propagation and disease control in Guadeloupe. *In* "Medio Siglo de Investigaciones Caneras en Cuba Congress" (INICA, ed.), pp. 4. INICA, Jovellanos-Mayari, Cuba.

Glaszmann, J.-C., D'Hont, A., and Feldmann, P. (1997). The genome of modern sugarcane varieties. *In* "Medio Siglo de Investigaciones Caneras en Cuba" (INICA, ed.), pp. 10. INICA, Jovellanos-Mayari, Cuba.

[1] Une copie est envoyée au service de documentation

Les qualités des travaux présentés ont été très hétérogènes selon l'origine et/ou le domaine concerné, un isolement scientifique marqué étant notable pour certaines stations avec de grandes difficultés pour obtenir les références bibliographiques nécessaires à certaines recherches.

Une expérience importante existe dans le domaine agronomique, par exemple pour l'étude des sols où la cartographie appliquée à la conduite des cultures (GPS et SIG), à l'irrigation et au choix variétal est progressivement étendue à l'ensemble du pays.

Des travaux importants sur l'irrigation et les problèmes de l'azote dans les vertisols sont effectués par la station de Villa Clara[2]. De nombreuses expérimentations dans tous les domaines sont effectuées dans les 13 stations et les 54 « blocs » expérimentaux répartis dans tout le pays.

L'amélioration de la canne de la canne à sucre

Les programmes de création variétale ont permis de sélectionner de nombreuses variétés locales (sigles JA, C ou CC) dont l'extension est passée de 30 % des surfaces en 1979 à plus de 90 % en 1997.

Certaines variétés ont été sélectionnées par mutagenèse (^{60}Co) ou par variation somaclonale. 9 somaclones sont cultivés commercialement sur plus de 2000 ha. Les caractéristiques recherchées concernent la résistance aux maladies avec des succès pour le charbon et la maladie des taches ocellées qui sert de modèle, la tolérance à la sécheresse ou aux sols salés et pour la teneur en sucre[3].

Les méthodes de sélection actuelle sont fortement orientées vers la maîtrise de l'interaction Génotype x Environnement qui est, à Cuba également, considérée comme étant le facteur clé en matière de sélection chez la Canne à sucre. A ce sujet, l'exception d'une variété à très large adaptabilité, JA 60-5, a été discutée pendant ce colloque. Le croisement à l'origine de ce clone a été renouvelé de nombreuses fois et des centaines de milliers de plantules produites et sélectionnées sans succès. Cette situation est à rapprocher de l'histoire des variétés NCo 310, NCo 376 et R 570. Ce clone présente un fonctionnement physiologique atypique pour une canne à sucre qui serait peut-être lié à son succès.

Micropropagation

Cuba possède plusieurs *biofabrica* spécialisées dans la production de vitroplants pour les pépinières. Celle visitée à Villa Clara prévoit de produire 5 millions de vitroplants pour sa troisième d'activité en 1997. La production de 1996 a été de 1,5 millions de plants par an avec 60 personnes employées.

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[2] Parmi les résultats exposés, on notera l'absence de réponse à l'azote en conditions hydromorphes et de compaction, l'incorporation de l'azote au centre de la souche et de manière enterrée et que la réponse à l 'azote est indépendante de la forme utilisée

[3] Le somaclone CC 82-105 présente une teneur en sucre améliorée et est cultivé sur plus de 1 000 ha. Sa sensibilité au charbon limite toutefois son extension future.

La méthode de micropropagation est rustique mais efficace. Aucune précaution particulière n'est prise pour le sevrage, les plants allant directement dans les plaquettes sous ombrière sans acclimatation. L'ombrière visitée présentait ainsi une mortalité des plants au sevrage de 10 à 15 %. La micropropagation se fait en milieu liquide avec un cytokinines (BAP 0,3 mg/l), l'enracinement nécessitant l'utilisation d'auxine (AIA).

Des essais de culture *in vitro* en immersion temporaire ont été exposés. Les taux de multiplication seraient augmentés d'un minimum d'un facteur 3 et jusqu'à 20 (!).

L'installation *in vitro* se fait par culture de « micro méristème » en faisant d'abord subir à de gros apex de 2 cm de long un traitement électrique (électrothérapie à 15 V / 15 A pendant 10 minutes). Ce traitement diminuerait la charge de contaminants. Les méristèmes prélevés ensuite ont une taille de 0,5 à 0,8 mm et permettent d'obtenir au bout de 6 à 10 mois plus de 10 000 plants chacun. 8 repiquages sont pratiqués au maximum.

Une communication présentée par une équipe cubaine (Peralta et al.) montrait que la culture *in vitro* ne débarrassait pas les plants de maladies, les taux de contaminations suivant étant trouvés dans des explants asymptomatiques :

RSD : 14,05 % des échantillons testés
LS : 10,6 %
SCMV et bacilliform virus : 6,7 %
Gommose : 5,76 %

La vitesse d'extension des pepinieres de vitroplants est impressionnante (de quelques milliers de vitroplants à plus de 14 millions en moins de cinq ans) mais le protocole appliqué conduit à éliminer de nombreux plants en début de phase de propagation en raison de problèmes phytosanitaires.

Une communication a présenté des travaux brésiliens sur l'application industrielle de la micropropagation de la canne à sucre.

Les intérêts trouvés pour cette méthode ont été, d'une part les gains de rendement considérables des parcelles issues de vitroplants (de 3-7 % à 32-38 % suivant les variétés) mais surtout l'économie considérable de boutures faite (de 47 à 50 %) par rapport aux pépinières classiques. Les pertes en plantation sont passées de 30 % pour les champs traditionnels à 0 % pour les vitroplants. Le périmètre sucrier concerné a calculé que ses besoins annuels de pépinières sont passés de 1276 ha à 450 ha grâce à l'utilisation de vitroplants.

Le CNIC, Centre National de la Recherche Scientifique

Une visite d'une journée a pu être organisée dans cet organisme de 1 000 personnes qui dispose d'une section de biotechnologie appliquée aux plantes, principalement à la canne à sucre.

Malgré de gros problèmes de moyens, la section Bioplantas de Maria Teresa Cornide et M. Ramos Leal effectue des travaux de qualité. Ils connaissent bien les travaux en biologie moléculaire de la canne à sucre du CIRAD et souhaitent très fortement une collaboration dans ce domaine.

Leurs travaux sur l'étude agronomique et moléculaire de variants résistants à la maladie des taches ocellés sont particulièrement convainquants ce qui est rare dans ce domaine (Ramos Leal et al., 1996. Plant Breeding 115, 37-42). Leurs réflexions sur les méthodes d'amélioration génétique de la canne et sur l'utilisation des biotechnologies sont avancées.

Le laboratoire de cryoconservation travaille actuellement peu sur la canne mais plus sur la Banane et les agrumes. Certains résultats obtenus indiquent le très bon comportement des apex de canne en cryoconservation avec absence de formation de cristaux de glace même en descente brutale en température. Les travaux « français » sont connus grâce à l'accueil il y a quelques années d'un chercheur à Montpellier sur la canne à sucre et à de régulières visites de F. Engelmann (ORSTOM/IPGRI). On peut noter qu'il n'avait pas été intégré que ces travaux avaient été effectués en grande partie au CIRAD (par F. Paulet). Ils sont attribués en totalité à l'équipe de l'Orstom de F. Engelmann.

Collaborations

Le désir de collaborations avec le CIRAD est très important, les responsables des deux structures (INICA et CNIC) ayant tenu à préparer des lettres d'intention pendant le colloque pour initier les échanges. L'étendue des collaborations envisageables n'est pas limitée même si plus de discussions ont eu lieu dans les domaines de l'amélioration des plantes (incluant la biologie moléculaire) et de la phytopathologie. La possibilité d'accueil de chercheurs ou techniciens en formation au CIRAD a été fréquemment abordée (en particulier en phytopathologie).

Afin de formaliser les collaborations possibles, les responsables de l'INICA et du CNIC ont précisé à plusieurs reprises qu'ils invitaient les personnes intéressées du CIRAD à venir leur rendre visite et qu'ils prenaient totalement en charge le séjour sur place à Cuba[4].

A l'occasion du colloque, des contacts ont été pris par plusieurs organismes non cubains intéressés par les travaux exposés par le CIRAD.

Le Directeur des Relations extérieures du GEPLACEA, E. Feilbogen souhaiterait une participation du CIRAD à une prochaine réunion du bureau des directeurs pour leur exposé l'état des connaissances en biotechnologie Canne à sucre.

Un responsable du Ministère des Affaires Etrangères du Mexique, Dr Luis Angel Rodriguez del Bosque (INIFAP) souhaiterait également une participation du CIRAD pour une réunion en Biotechnologie.

[4] Le coût du billet d'avion de Guadeloupe à Cuba est de 2300 F (1 vol par semaine)

Conclusion

Cuba est un grand pays sucrier présentant des potentiels de recherches en Canne à sucre très importants. Des solutions originales à différents problèmes de culture de la canne à sucre ont été trouvées compte-tenu des contraintes économiques du pays (peu d'intrants, forte utilisation de la lutte biologique par exemple). Un désir d'ouverture fort vers l'extérieur est constamment exprimé. Les activités du CIRAD sont parfois assez bien connues (au CNIC par exemple) et des collaborations vivement souhaitées par les instituts cubains.

Dans un premier temps, vu l'étendue des collaborations possibles, l'INICA et le CNIC sont prêts à accueillir et à prendre en charge le séjour sur place des personnes du CIRAD concernées pour définir plus précisément les points de collaborations possibles.

Annexes

Annexe 1 : programme du congrès

Annexe 2 : Feldmann, P., Rott, P., Oriol, P., Paulet, F., Daugrois, J., and Sapotille, J. (1997). Use of tissue culture of sugarcane for rapid propagation and disease control in Guadeloupe. *In* "Medio Siglo de Investigaciones Caneras en Cuba Congress" (INICA, ed.), pp. 4. INICA, Jovellanos-Mayari, Cuba.

Annexe 3 : Glaszmann, J.-C., D'Hont, A., and Feldmann, P. (1997). The genome of modern sugarcane varieties. *In* "Medio Siglo de Investigaciones Caneras en Cuba" (INICA, ed.), pp. 10. INICA, Jovellanos-Mayari, Cuba.

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Bienvenida

El Instituto Nacional de Investigaciones de la Caña de Azúcar (INICA) les da la más cordial bienvenida en ocasión de celebrarse el 50 Aniversario de la Fundación de la Estación Provincial de Investigaciones de la Caña de Azúcar de Jovellanos, provincia de Matanzas, y el 45 Aniversario de la Estación Provincial de Investigaciones de la Caña de Azúcar de Mayarí, provincia de Holguín, ocasión en que se organiza este evento científico en conmemoración de tan significativos acontecimientos, con los objetos de:

1. Realizar un balance del desarrollo alcanzado por las investigaciones cañeras en Cuba durante los últimos 50 a.
2. Propiciar el intercambio de experiencias entre investigadores cañeros nacionales y de diferentes paises.
3. Definir temas de interés común para la cooperación internacional y la transferencia de tecnologías entre las naciones productoras de caña, básicamente del área latinoamericana y del caribe.

Les deseamos una provechosa participación en el evento y que disfruten de la estancia en nuestro país.

Información General

1. Las inscripciones se realizarán en la sede donde se desarrollará el Evento, de 8:00 a 9:30 a.m. en Jovellanos el día 23 de junio.
2. Las inscripciones en la sede de Guaro se realizarán en igual horario el día 26 de junio.
3. Los talleres de Mejoramiento Genético y Biotecnología y los de Plagas y Enfermedades se realizarán en el salón teatro de la Estación de Jovellanos.
4. Los talleres de Sostenibilidad de Agroecosistemas y Manejo Agrícola de las plantaciones cañeras se realizarán en el salón de actos de la Estación Provincial de la Caña de Azúcar de Guaro.
5. Se ubicarán 2 exposiciones científico-técnicas:
   Área Biológica en Jovellanos
   Área Tecnológica en Guaro
6. Los trabajos en pósters se ubicarán en áreas aledañas a las exposiciones en ambas sedes.
7. Los servicios y necesidades de los participantes serán resueltos por la Mesa de Secretaría del Evento en cada sede.
8. Cualquier información adicional se deberá solicitar a la Comisión Organizadora del Evento en cada sede.

Reglamentaciones del Evento

1 Para asistir a las sesiones técnicas y actividades programadas se requerirá el distintivo del evento
2 Las ponencias y discusión el los talleres se deberán ajustar al tiempo establecido en el horario previsto.
3. Las preguntas o comentarios versarán, exclusivamente, sobre el tema en discusión.
4. Durante las sesiones el ponente deberá acatar las indicaciones del moderador, en particular lo referente al tiempo.
5. Los ponentes que vayan a utilizar diapositivas, acetatos o demos deben entregar el material al oficial de sala con anticipación.
6. A fin de garantizar el desarrollo del Programa se agradece la más puntual asistencia.

Programa General

Domingo 22 de junio

8:00 a.m. a 12 m.	Recibimiento de delegados nacionales y extranjeros

Lunes 23 de junio

8:00 a.m. a 9:00 a.m.	Inscripción en la Sede de Jovellanos
9:00 a.m. a 10:00 a.m.	Ceremonia de apertura
10:00 a.m. a 10:15 a.m.	*Receso*
10:15 a.m. a 12:00 m.	Taller sobre plagas y enfermedades
12:00 m. a 12:15 p.m.	*Receso*
12:15 p.m. a 2:00 p.m	Continuación del Taller de Plagas y Enfermedades
2:00 p.m. a 3:00 p.m.	*Almuerzo*
3:00 p.m. a 5:00 p.m.	Discusión de pósters de Enfermedades, Plagas y Biotecnología. Recorrido por la Estación
5:00 p.m. a 6:00 p.m.	Cocktail de bienvenida

Martes 24 de junio

9:00 a.m. a 11:00 a.m.	Taller de Genética
11:00 a.m a 11:15 a.m.	*Receso*
11:15 p.m a 1:45 p.m.	Continuación del Taller de Genética
2:00 p.m. a 3:00 p.m.	*Almuerzo*
3:00 p.m. a 5:00 p.m.	Discusión de pósters de Genética

6:00 p.m.	Acto de clausura
7:00 p.m.	Cena de despedida

Miércoles 25 de junio

7:00 a.m.	Salida para la provincia de Villa Clara
9:30 a.m.	Visita a la Estación Provincial de la Caña de Azúcar de la provincia de Villa Clara
10:30 a.m	Visita al Centro de Información y Referencia de Suelos
	Receso
10:45 a.m. a 12:00 m.	Visita a la Biofábrica de Caña de Azúcar
12:30 p.m.	*Almuerzo*
1:30 p.m.	Salida para la provincia de Holguín
8:30 p.m.	Cena y alojamiento en el Hotel Pernik, en la ciudad de Holguín

ueves 27 de junio

8:00 a.m.	Salida para la Estación Provincial de Mayarí
8:30 a.m a 9:30 a.m.	Llegada a la Estación de Mayarí e inscripción en el Evento
9:30 a.m. a 10:00 am	Inauguración de la Exposición Científico-Técnica del Área Tecnológica
10:00 a.m. a 10:30 a.m.	Palabras de bienvenida
10:30 a.m. a 10:45 a.m.	*Receso*
10:45 a.m. a 1:00 p.m.	Taller de Sostenibilidad de Agrosistemas
1:00 p.m. a 2:00 p.m.	*Almuerzo*
2:00 p.m. a 4:30 p.m.	Visita de campo
	Demostraciones:
	1. Preparación de suelos
	2. Fertilización
	3. Elaboración de compost
	4. Cosecha en verde
4:30 p.m.	Inauguración de Biofábrica
5:00 p.m.	Salida para el Hotel Pinares de Mayarí
9:00 p.m.	Actividad nocturna

Viernes 28 de junio

9:00 a.m.	Taller de Manejo de Agrosistemas
10:30 a.m.	*Receso*
11:00 a.m. a 12:00 m.	Discusión de pósters
12:00 m. a 1:30 p.m.	Visita a exposición permanente
1:30 p.m.	Acto de clausura
2:00 p.m.	Almuerzo de despedida

Programa

50 Aniversario de la Estación Experimental de la Caña de Azúcar de Jovellanos

45 Aniversario de la Estación Experimental de la Caña de Azúcar de Mayarí

23 al 27 de junio de 1997
Jovellanos, provincia de Matanzas
Guaro, provincia de Holguín
Cuba

USE OF TISSUE CULTURE OF SUGARCANE FOR RAPID PROPAGATION AND DISEASE CONTROL IN GUADELOUPE

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Abstract. Micropropagation of sugarcane has been used in Guadeloupe for ten years for the establishment of disease-free nurseries. Micropropagation is used for production of healthy seed of established varieties, mainly to control Leaf Scald Disease and Ratoon Stunting Disease, and to increase the rate of propagation of new varieties. A project for establishing clean nurseries has been developed by CIRAD in collaboration with the Interprofessional Technical Center for Cane and Sugar (CTICS). The system consists of three successive steps: nurseries of elite clones (20,000 tissue-cultured plants representing ten clones and a total surface of 2 ha/year), secondary nurseries (30 ha) and finally commercial nurseries (200-300 ha). In this way, 2 to 3000 hectares can be planted within 24 months. CIRAD ensures the genetic conformity and sanitary quality of the plant material at each step until planting in commercial fields. In order to minimize the risks of somaclonal variation, growth substances are not added to the culture media. For the same reason, bud culture is the preferred method because recurrent callogenesis or "true" meristem culture has resulted, in a few cases, in deleterious variations. This project can be adapted and modified according to specific needs. For example, it should be possible to reduce the number of nursery cycles to avoid rapid reinfection of varieties by pathogens, or when the urgent replanting of a large area is necessary. It has recently been used for the propagation and export to French Guyana of thousands of plantlets (63 new varieties) in a short period of time. To guarantee the absence of diseases, plantlets were propagated with material issued from the *in vitro* collection maintained at CIRAD, Montpellier. This *in vitro* collection was established following the release of the clones from the sugarcane quarantine.

Introduction. Sugarcane crops are set up for several years. However, yields in sugar decrease progressively after a few cycles due to aging of the stool, accumulation of pests and systemic diseases such as Leaf Scald and Ratoon Stunting Disease. Crop productivity is maintained by regular replanting of perfectly healthy, vigorous cuttings taken from clones of proven suitability.

Sugarcane culture possesses several characteristics which make it vulnerable to diseases:

1. the nature of sugarcane production reduces the opportunity to replace one variety with another
2. the use of cuttings for plantings facilitates the dissemination of pathogens
3. due to the clonal nature of sugarcane, there is a lack of genetic diversity in a given large growing area, and this favors the spread of epidemics.

The problem of disease necessitates constant attention in production zones and disease control consists essentially of preventive measures. Prevention is aided by a better knowledge of the pathogens, their epidemiological characteristics and their potential effects on yield. It is most important to be able to replant without introducing new diseases, therefore a reliable quarantine system and an ability to dispense clean varieties is required. Finally all of this supposes a capacity for rapid diagnosis and tools for diagnosis which are usable under field conditions. The producers use cuttings obtained from specific nurseries, the conditions of which must guarantee varietal purity, absence of diseases and rapid and uniform growth. The basic plant material of these nurseries is generally made up of hot-water treated cuttings taken from mother-stock nurseries. Long hot-water treatments, widely used to decontaminate sugarcane clones of various systemic diseases, do however present some inconveniences. It is difficult to perform on large volumes of cuttings. Moreover, certain varieties do not tolerate long treatments and certain pathogens are only partially destroyed.

Today, tissue culture techniques have been developed for the propagation of sugarcane (Guiderdoni *et al.*, 1995) and have overcome these limitations.

Materials and Methods.

Obtention of disease-free varieties.

They are obtained from the quarantine service of CIRAD in Montpellier, France. It responds to the growers' demands for sugarcane cuttings of excellent agronomic quality and guaranteed health. The condition of the cuttings distributed conforms to the recommendations of the International Plant Genetic Resources Institute and the International Society of Sugar Cane Technologists (Frison and Putter, 1993).

The plants are observed and treated with fungicides and insecticides and hot-water treated for two crop cycles. Tests to detect viral and bacterial diseases are also conducted. Immunofluorescence tests using a microscope equipped for epifluorescence allow the identification of the bacterial pathogens responsible for Leaf Scald and Ratoon Stunting Disease. These two diseases are considered as the most important to be controlled in the conditions of Guadeloupe (Rott and Feldmann, 1991; Rott *et al.*, 1995).

The clones are then sent by air as tissue-cultured plants to the sugarcane propagation laboratory of CIRAD in Guadeloupe after being installed in vitro by bud or meristem culture.

Micropropagation.

The developed techniques involve the tissue culture of explants, buds and true meristems, 300-500µ size, taken from sugarcanes having undergone two years of quarantine. Plants propagated from these tissues retain the seal of sanitary guarantee of the quarantine. Surface desinfection with alcohol followed by flame burning is processed before sampling of young bud explants or apical meristem.

The tissue culture is initiated in Petri dishes using Murashige & Skoog medium (MS) modified with Fiji vitamins, 2.5 g/l Gelrite as gelling agent, active charcoal to control browning and 30 g/l sucrose. PH is adjusted to 5.6. Propagation is carried out by natural tillers formation *in vitro* using MS liquid culture media with high level of sucrose (50g/l).

Transferring

The well rooted plantlets are transferred in small peat pellets after removing excess leaves and roots and dipping in fungicide (Phosetyl-Al.). They stay 2-3 days under shade and high humidity level before growing in the nursery greenhouses. In the nursery, the plantlets are grown under greenhouse for 1 month, following a proven protocol and then transferred to the field (Paulet et al., 1991). The first cuttings aimed at commercial nurseries or production plots are taken from these plants after an average of eight months' growth.

Results and Discussion.

Rapid propagation of sugarcane in Guadeloupe.

Micropropagation of sugarcane has been used in Guadeloupe for ten years for the establishment of disease-free nurseries (Feldmann and Rott, 1991; Feldmann *et al.*, 1991). Micropropagation is used for production of healthy seed of established varieties, mainly to control Leaf Scald Disease and Ratoon Stunting Disease, and to increase the rate of propagation of newly released or introduced varieties. A plan for establishing clean nurseries has been developed by CIRAD in collaboration with the Interprofessional Technical Center for Cane and Sugar (CTICS). The system consists of three successive steps: nurseries of elite clones (20,000 tissue-cultured plants representing ten clones and a total surface of 2 ha/year), secondary nurseries (30 ha) and finally commercial nurseries (200-300 ha). In this way, 2 to 3000 hectares can be planted within 24 months. Each agreed variety is propagated through this scheme. CIRAD ensures the genetic conformity and sanitary quality of the plant material at each step until planting in the commercial fields. This plan can be adapted to other situations (national propagation systems, research centres, agro-industrial perimeters) and modified according to the needs. For example, it should be possible to reduce the number of nursery cycles to avoid rapid reinfection of varieties by pathogens, or when an urgent replanting of large areas is necessary.

In vitro storage.

As well as classic field maintenance, two techniques have been developed for the preservation of collections. The tissue culture collection contains healthy sugarcane plants stored over the short or medium term *in vitro*. They are used to launch mass micropropagation at any time. Cryopreservation allows the long term safeguard of sugarcane apices, frozen in liquid nitrogen, with a view to preserving genetic resources (Paulet *et al.*, 1993).

Tissue culture.

The use of MS liquid culture media has enabled plant multiplication rates of 3-5 per month to be achieved, which represents a potential, starting with a few individuals, of several hundred thousand plantlets (called "tissue-cultured plants") per year. No antibiotics or growth substances are added to the culture media, in order to minimize the risks of somaclonal variation. Physiological rejuvenation due to the passage in tissue culture leads sometimes to an increase in tiller formation, which can be up 50% greater than in the original material, whilst the diameter of the shoots reduces. This behaviour presents the advantage of increasing the factor of multiplication. It tails off and disappears at the end of propagation.

However, the monitoring of tens of thousands of plants has revealed only two cases where this type of modification persisted.

After propagation of hundreds of different genotypes, a very low level of somaclonal variation has been observed. One somaclone of Co6415 had shown in 1986 a drastic increase of tillers and decrease of stalk diameter after micropropagation. This somaclone had been propagated after recurrent callogenesis. Since that, we do not use any growth substance in our tissue culture media. In another case, the clone B8008 showed after true meristem culture a stunting growth that was undesirable. We have yet to be careful in using true meristem culture in sugarcane. Stability of propagated plantlets has to be checked before plantation in nurseries. Nevertheless, such modifications have remained very rare and other propagated subclones of these two varieties never showed any variation.

Tissue culture enables rapid propagation of healthy varieties, protected from pathogens and the uncertainties of climate. The introduction of new varieties to a growing area can then be made, with maximum sanitary guarantees, by way of a small parcel sent by mail. For example, thousand of plantlets of 63 disease-free clones have been recently propagated for export to French Guyana in an attempt to avoid the spread of Leaf Scald and Ratoon Stunting Disease in this area. The use of in vitro propagation of plantlets stored in vitro after quarantine has allowed to accelerate the time of introduction. Three years of quarantine and nursery have been saved in this operation.

The current development of cryopreservation will permit to respond quickly to the need for propagation of a higher number than the 200 of disease-free clones today maintained in in vitro collection.

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THE GENOME OF MODERN SUGARCANE VARIETIES

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Abstract.
Molecular markers provide a powerful tool to study the structure and variation of the sugarcane genome. Diversity has been studied using DNA Restriction Fragment Length Polymorphism (RFLP) revealed within the cytoplasmic genomes and within the nuclear genome, using ribosomal repeated DNA sequences and single copy sequences as probes. This permitted confirmation of previous taxonomic schemes and provided novel information. Genome mapping studies have been focussed on modern varieties for a better understanding of their complex genomic structure. This permitted the construction of a detailled linkage map and permitted investigation of some aspects of chromosome inheritance and pairing behaviour in varieties. As a complement, DNA *in situ* hybridization on chromosomes allowed assessing the contribution of ancestral species to the genome of modern breeding materials. Comparative mapping with distant diploid relatives revealed a large amount of chromosome structure conservation between species, especially between sugarcane and sorghum, and to a smaller extent between sugarcane and maize. Our first results concerning linkage between markers and genes of agricultural interest are encouraging regarding the future use of molecular markers in breeding schemes.

Introduction.
First cultivated *Saccharum officinarum* clones have been replaced in the 1920s by materials introgressed by wild species, mainly *Saccharum spontaneum* . Since then, most cultivated sugarcane varieties have kept this hybrid and aneuploid genomic structure: approximately a hundred *S. officinarum* chromosomes associated with a few *S. spontaneum* chromosomes.
The emergence of new techniques based on molecular biology brought about new tools for the analysis of the sugarcane genome; in particular, the socalled Restriction Fragment Length Polymorphisms (RFLPs) enable the detection of numerous genetic loci and their mapping within the genome through linkage analysis. Due to the high ploidy level, molecular genetic markers cannot be applied to sugarcane as they are to most other plants. For identifying a linkage between two loci, it is desirable that both produce a "Single Dose Restriction Fragment" (SDRF) *i.e.* a fragment produced by a single copy among all the homologous loci (Wu *et al.* 1992) and which will therefore segregate as a monogenic marker. An ideal mapping population will therefore display a high number of SDRFs, and show strong linkage disequilibria.
An elegant way to improve the mapping population is to take advantage of the derivatives of androgenetic doubled haploids available for the *S. spontaneum* clone SES208 (Moore and Irvine, 1991). This approach enabled construction of a linkage map based on RFLPs including 212 markers distributed on 39 linkage groups (da Silva et al., 1993) and a map based on AP-PCR including over 230 fragments (Al-Janabi et al., 1993).

Researchers at CIRAD developed another approach inspired by earlier isozyme analyses (Glaszmann *et al.*, 1989; Eksomtramage *et al.*, 1992) and tried to characterize the interspecific aneuploid genome structure of modern varieties. Particular lines of research developed are:
- the study of genetic diversity among varieties in relation to ancestral species;
- the mapping of the genome within modern varieties, with special reference to the relationships between the various subgenome that are coexisting;
- the comparison of sugarcane genome maps to those of related diploid relatives such as maize and sorghum;
- the development of molecular cytogenetic techniques for addressing the interspecific origin of modern breeding materials, and;
- exploratory applications of molecular markers to assist conventional screening techniques.

Surveys of cytoplasmic and nuclear polymorphisms for a better knowledge of genetic diversity in sugarcane
Genetic diversity revealed with RFLPs was analysed in a sample of 50 clones representing various taxonomic groups of the *Saccharum* complex using heterologous probes. Two probes were chloroplastic and 9 were mitochondrial (D'Hont et al., 1993), one represented a repeated nuclear sequence covering ribosomal DNA (Glaszmann et al., 1990) and 33 represented low copy nuclear sequences (Lu et al., 1994a). The results confirmed previous taxonomic schemes derived from morphological, cytological and biochemical data, and brought about novel information (Fig. 1) :
- mitochondrial diversity was observed between and within *Saccharum spontaneum* and *S. robustum* ; diversity is larger in *S. spontaneum* ; *S. robustum* displays two types, one of which characterizes all *S. officinarum* clones ; this allows excluding part of *S. robustum* clones from potential ancestors of *S. officinarum* ;

- rDNA diversity showed :
. an opposition between the Indian forms and the large chromosome numbered southern forms within *S. spontaneum* ;
. the presence in weak dosage in *S. officinarum* of markers of the southern *S. spontaneum* forms.
- diversity revealed with low copy nuclear sequences showed the same geographic structure within *S. spontaneum*.

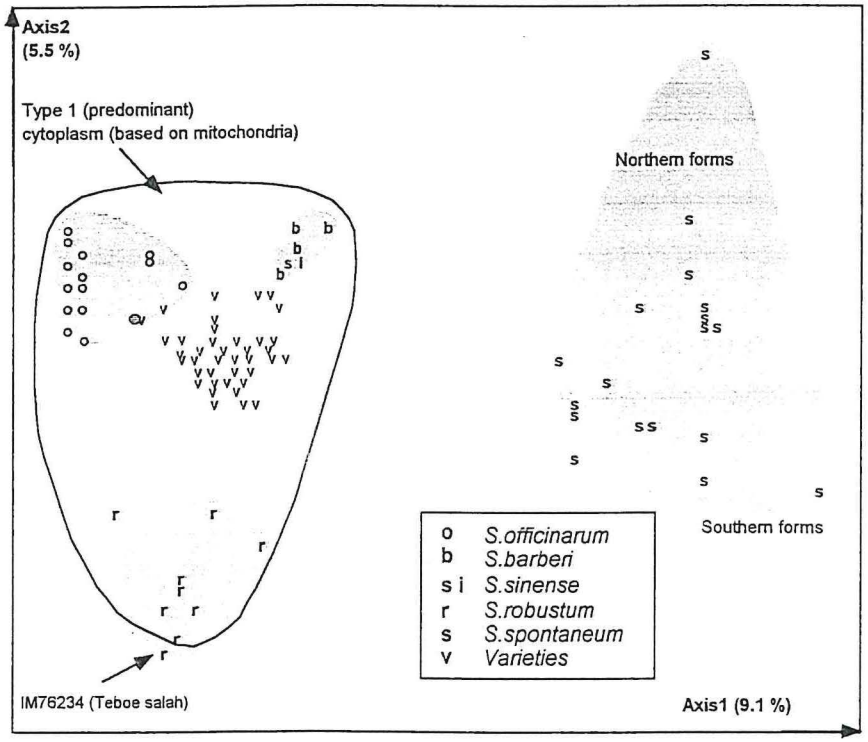

Figure 1: Distribution of the sugarcane clones in plane (1-2) of the factor analysis of correspondences among 463 polymorphic bands obtained with 13 maize nuclear probes. Large diversity within the *S. spontaneum* species tends to distinguish northern from southern forms. Clones of *S. officinarum*, *S. barberi*, *S. sinense* and modern varieties display the same cytoplasm as the dominant type of *S. robustum* (from D'Hont et al., 1993 and Lu et al., 1994b).

The nuclear probes were then applied to 40 commercial varieties (Lu et al., 1994b). Besides providing an exceedingly powerful identification tool, these probes allowed characterizing the fraction of the *S. spontaneum* genome still present in modern varieties.

These findings will help broaden the genetic base of modern varieties and foster the exploitation of the diversity available in germplasm collections.

Analysis of modern variety genome through genetic mapping

We first conducted a preliminary study, with the RFLP analysis of 32 individuals derived from selfing of variety SP701006 of COPERSUCAR, using maize DNA probes covering the whole maize genome (D'Hont et al., 1994). Strong cosegregations allowed placement of 94 markers into 25 cosegregation groups. Eighteen of these groups involved *S. spontaneum*-specific markers and were thus assumed to correspond to chromosomes initially contributed by the wild species. Cosegregation groups could be assembled into 7 linkage groups on the basis of probes in common. A large degree of synteny between sugarcane and maize could be inferred, with a much lower rate of recombination in sugarcane.

We then undertook a larger scale mapping study using the self progeny of R570, an elite variety produced by the Centre d'Essai, de Recherche et de Formation (CERF) (Réunion) (Grivet et al., 1996) . It enabled us to confirm previous results and it widened the general understanding of genome perspectives in sugarcane. A set of 128 maize and sugarcane RFLP probes and two isozymes were used, so that 428 markers were placed onto 99 cosegregation groups, based on linkages in coupling only. The number of simplex alleles that could be detected per locus averaged 4, but, in some cases, as many as 10 alleles were revealed. The cosegregation groups, that can be considered as chromosomes or chromosome regions, could tentatively be assembled into 10 basic linkage groups on the basis of common probes. The compositions of the linkage groups fitted very well the ones detected in our previous mapping study on the self progeny of variety SP701006 (D'Hont et al., 1994).

Markers specificity was investigated for 61 probes and two isozymes, leading to identification of 84 *S. officinarum* and 69 *S. spontaneum* inherited markers. Their distribution in cosegregation groups showed a better map coverage for minority *S. spontaneum* chromosomes, which is consistent with our previous mapping study (D'Hont et al., 1994) and can be related to the results of diversity studies (Eksomtramage et al., 1992; Glaszmann et al., 1990; Lu et al., 1994a; 1994b). It also permitted detection of the occasional presence of recombinations between the two basic genomes, which contradicts the usual assumption that interspecific intrachromosomal recombination is absent (Price, 1965).

A few repulsions between mapped homologous markers showed overall random pairing between chromosomes typical of autopolyploids with, however, preferential pairing between *S. spontaneum* chromosomes (Fig. 2).

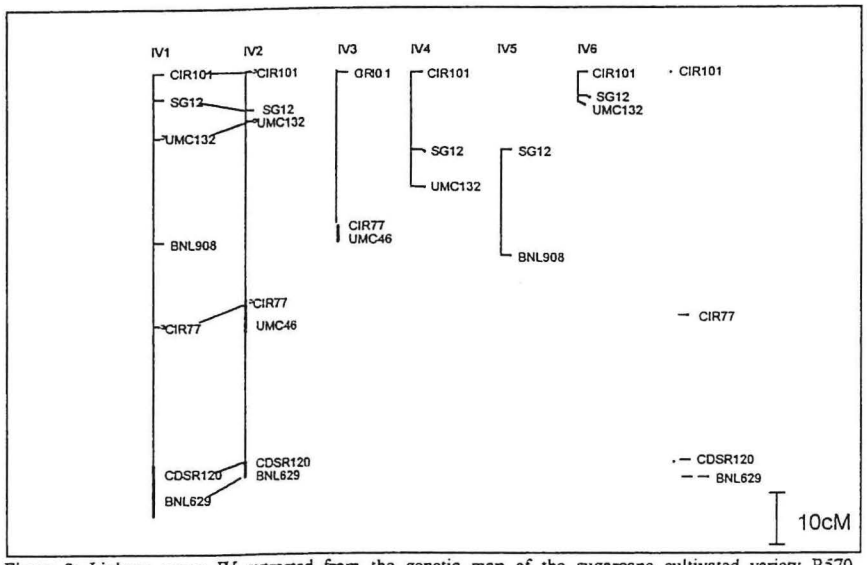

Figure 2: Linkage group IV extracted from the genetic map of the sugarcane cultivated variety R570. Cosegregation groups, corresponding to linkages between markers in coupling, are represented by vertical bars. Uncertain orders are represented by a bold line. Linkages in repulsion between two markers generated by the same probe are indicated by horizontal dashed lines. When the parental specific origin of a marker is known, it is indicated either with a black circle for *S. officinarum* or a white lozenge for *S. spontaneum*. Unlinked markers are represented by a dash or the corresponding symbol (black circle or white lozenge) if their origin is known (from Grivet et al., 1996).

No definite structural differences were detected between homoeologous chromosomes of *S. officinarum* and *S. spontaneum*, although it was suspected in one linkage group (group VIII). It seems that differences, if they exist, involve only simple cytogenetic rearrangements.
Considering that recombination between chromosomes of both origins may be possible and that no definite structural difference was detected between chromosomes of both ancestral species, a tentative *Saccharum* map was constructed by pooling linkage information for each linkage group. This permitted proposing a first core map of sugarcane, which will be of great use for further investigation with molecular markers in sugarcane breeding.

Identification of parental chromosomes in intergeneric and interspecific hybrids by genomic DNA *in situ* hybridization
Since introgression with wild relatives, mainly *S. spontaneum* and *E. arundinaceus*, is essential to sugarcane improvement, we have developed *in situ* hybridisation in order to characterize introgressed materials in sugarcane intergeneric and interspecific hybrids. We first showed the possibility using comparative genomic *in situ* hybridization to differentially detect the chromosomes from *S. officinarum* and *E. arundinaceus* in a hybrid between these two genera (D'Hont et al., 1995).

The ability to differentiate the chromosomes from *S. officinarum* and *S. spontaneum* in interspecific hybrids between these two species was then demonstrated using the same approach (D'Hont et al., 1996).
This technique allows the visualisation of the *S. spontaneum* contribution to modern sugarcane varieties. In variety R570, we have identified whole chromosomes originating from *S. spontaneum* and recombinant chromosomes between *S. officinarum* and *S. spontaneum*. It confirmed the results based on RFLP mapping and provided a quantification of the amount of interspecific recombination.

Comparative mapping with related species; new perspectives for sugarcane breeding
All species that compose the primary gene pool of sugarcane (*Saccharum* and a few closely related genera) are polyploid. At the tribe level, maize and sorghum are two important diploid crops that have received much attention from molecular geneticists. Comparative mapping between sugarcane and those two crops has recently progressed with the development of common mapped probes that serve as bridge-loci to identify syntenic (i.e. of similar chromosome constitution) genome regions in the three plants.
The sugarcane map of R570 described above had 84 common loci with a sorghum map developed at CIRAD on two recombinant inbred lines populations and including 199 loci. Comparison of the distribution of bridge-loci in the two maps showed extensive homology and, in most cases, the conserved linkage groups showed a strong colinearity (Dufour et al., in press) (Fig. 3).
Comparison with published maize maps (Burr and Burr, 1991, Gardiner et al., 1993) revealed that most of sugarcane and sorghum linkage groups corresponded to two or more paralogous maize chromosomes segments (Grivet et al., 1994, Dufour et al., 1996).
Synteny may allow the identification of orthologous (derived from a single ancestral gene through speciation) quantitative trait loci for homologous agronomic traits among the three crops. Having diploid model crop relatives could be of great benefit for a better understanding of trait genetics in sugarcane.

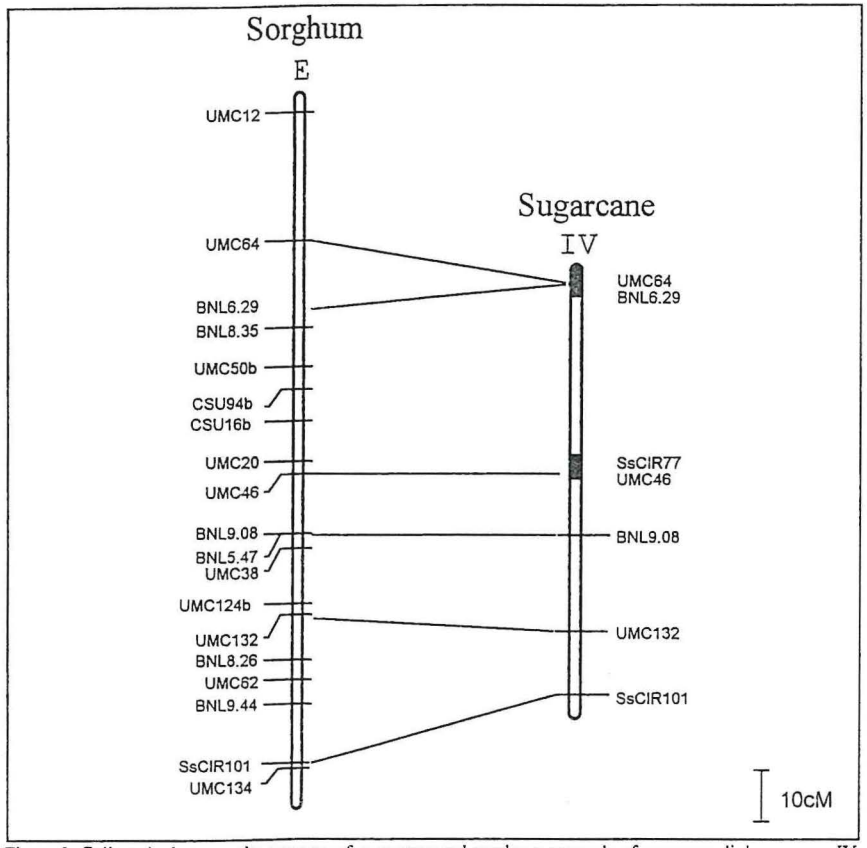

Figure 3. Colinearity between the genomes of sugarcane and sorghum; example of sugarcane linkage group IV (from Dufour et al., 1996).

Starting to mark favorable traits in modern varieties

Genetic mapping is the starting point for exploring the possible use of molecular markers in breeding schemes. The second step is the identification and localisation on the map of genes which control variation in important traits. For that purpose, we undertook a preliminary field experiment study on a subset of selfed progeny individuals of R570 that were used for mapping. They were scored for several selection criteria such as resistance to rust disease (65 individuals) and several sugar yield components (58 individuals). Since segregating markers correspond to dominant alleles due to random pairing, the segregating population was divided into two classes for each marker (presence *vs* absence). Significant differential expression of a quantitative trait between the two marker classes was interpreted as indicative of a linkage between the marker and a Quantitative Trait Locus (QTL).

Rust resistance was evaluated through both field experiments and greenhouse tests with artificial inoculation. Field scores were very well correlated between the plant and first ratoon crops and were in good agreement with greenhouse tests. The data showed a 3 to 1 segregation ratio in the progeny, which indicates the possible presence of a major resistance gene in R570. This mendelian inheritance had already been suggested by Saumtally et al. (1994). This putative major gene accounted for more than half the phenotypic variation in the progeny and was strongly linked (10 recombination units) to an independent marker of the map; this was further tested with a larger progeny (Daugrois et al., 1996) (Fig. 4).

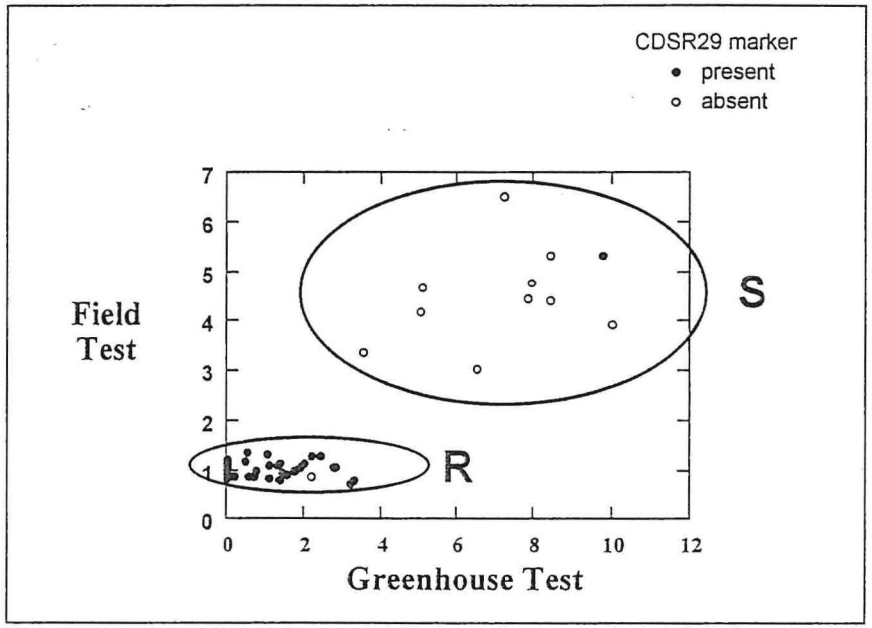

Figure 4: Distribution of R570 selfed progeny individuals on a diagram reporting both field (first ratoon, R94) and greenhouse rust scores. In both cases a high score means high sensitivity. This permits to visualize the clear-cut segregation for this character and the tight linkage with marker CDSR29H5 (from Daugrois et al., 1996).

Quantitative trait loci were found for almost all sugar yield components investigated (stalk height, stalk diameter, tillering, brix). These results have to be taken with care regarding the small size of the progeny evaluated. Larger scaled experiments have to confirm those preliminary results. Nevertheless, this shows that identification of QTL may be possible despite the high ploidy of cultivated sugarcane clones; molecular markers could thus be an interesting tool for assisting classical selection procedures in sugarcane breeding.

Given the contrasting resolution of the respective ancestral genomes on the map, concentrating efforts on the easily accessible *S. spontaneum* part of the genome seems a promising strategy for identifying QTLs of interest in breeding. Indeed, *S. spontaneum* has brought many useful genes to modern varieties along a limited and well marked genome portion. The same will be true for *Erianthus* if the corresponding current breeding efforts in various research stations are successful.

Conclusion

The latest three years have seen very significant breakthroughs with the production of several sugarcane genome maps, the demonstration of wide similarities with maize and sorghum genomes, and the improved understanding of genome balance in modern varieties thanks to molecular markers and molecular cytogenetic techniques. The availability of a panel of new molecular tools will enhance interspecific and intergeneric introgression, which is the key to further genetic improvement in sugarcane.

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