RESEÑA

MOLECULAR CARACTERIZATION AND IDENTIFICATION OF *SACCHARUM* COMPLEX CLONES FOR BROADENING THE GENETIC BASE OF SUGARCANE

M.C., Ing. Orlando Coto Arbelo
Departamento de Bioplantas, Centro Nacional de Investigaciones Científicas, Avenida 25 y Calle 158, Playa, Apartado Postal 6414, Ciudad de La Habana, Cuba.

PRSENTED IN OPTION TO DEGREE OF PH.D. IN AGRICULTURAL SCIENCES

The knowledge about the amount and distribution of the genetic variation among cultivated species and their wild relatives is necessary to improve the efficiency of breeding and genetic conservation programs.

Sugarcane belongs to the *Saccharum* genus, Andropogoneae Tribe of the Grass family. Six species are included in this genus characterized by a high ploidy level: *Saccharum spontaneum* L. (2n = 64-112) is a wild highly polymorfic species, *Saccharum robustum* Brandes & Jeswiet (2n = 60-80) a wild species also, *S. officinarum* L. (2n = 80) is a high sugar producer, *S. barberi* Jeswit (2n = 111-120) and *S. sinense* Roxb. (2n = 81-124) are presumed to be of interspecific origin, involving *S. officinarum* and *S. spontaneum* crosses (Sreenivasan et al., 1987). On the other hand, *S. edule* is considering as a marginal form of sugarcane, cultivated as vegetable.

A major limitation of sugarcane breeding is its reduced genetic base, modern cultivars being descendants of a few ancestors or foundation clones. Their cytoplasmic origins are more limited and are mainly represented by 3-5 *Saccharum officinarum* clones.

The intercrossing among the members of the so called *Saccharum* complex points to them as the most probable sources of new genetic variation for sugarcane introgression.

Most wild and cultivated species of the *Saccharum* complex which are known to be involved in the origin of sugarcane are under serious threat of genetic erosion in their natural habitats.

Regular germplasm collections exhibit gaps in the representation of clones from geographical regions of the South East Asia and continental Africa. Among the regions considered higher priorities to exploration and collection are Laos and Vietnam. Thus, characterization, identification, conservation and management of wild relatives and ancestors are main objectives of research associated to plant breeding programs.

These research is based on that molecular markers have added a powerful new dimension to genetic studies and to the management of plant genetic resources for plant breeding and they could be very valuable tools to study genetic relations in the *Saccharum* complex by evaluating polymorphism at the level of DNA.

The main objectives of our work was to evaluate the nuclear and cytoplasmic genetic diversity by Restriction Fragment Length Polymorphism (RFLP) of the sugarcane germplasm collections currently used in the cuban introgression program, to determine the use such cytoplasmic polymorphism for the taxonomic identification of *Saccharum* complex clones, to recommend appropriated markers (RFLP and PCR by specific primers) for the identification of species and clones studied and to assist sugarcane breeders in preservation, efficiently utilizing and to complete this resources.

In the aim to get the former objectives, a set of four collections were studied:

- the foundation clones (Collection 1), which constitute the reduced genetic base of sugarcane breeding, two cuban commercial hybrids were included as references. The genetic diversity was evaluated by RFLP using single copy probes from maize.
- Original forms of known taxonomic origin (Collection 2), studying the nuclear and cytoplasmic genetic diversity of the *S. officinarum* clones, which represent the female genetic base used in Cuba. In the cytoplasmic study a group of 11 new clones collected in Laos with an outstanding behavior due to the high vigor and resistance to diseases were included and for determining the utility of the cytoplasmic diversity for the identification of these kind of clones. The nuclear genetic diversity was surveyed using both homologous and heterologous probes and the cytoplasmic study was conducted also by RFLP using heterologous probes.
- Wild forms of unknown origin (Collection 3), these clones were collected in five localities of Laos (ECL, Expedition Cuba-Laos). Foundation clones from *S. spontaneum* and *S. officinarum* were included as references. Homologous and

heterologous probes from maize were used. A taxonomic identification of the clones was assessed using *Miscanthus*, *Erianthus* and 5S rDNA specific primers.

- A core collection (Collection 4), consisting of 89 outstanding clones, which were selected from 269 accessions previously evaluated for brix, plant height, the stalk weight components and resistance to smut (*Ustilago scitaminea* Sydow), rust (*Puccinia melanocephala* H. y P. Sydow) and Sugarcane Mosaic Virus (SCMV) in four localities. The group markers and the taxonomic identification by 5S rDNA specific primers reported in the previous collection were confirmed.

All the plant materials were obtained from the germplasm bank of the National Institute for Sugarcane Research (INICA).

The results obtained in the foundation clones collection permitted to detect that the greatest diversity among the species surveyed was shown by *Saccharum spontaneum* clones and the genotypes of *Erianthus* spp. compared with *Saccharum officinarum*. The *S. spontaneum* and *Erianthus* clones are the main genotypes used to introduce novel genetic variability during the nobilization in sugarcane for breeding the agronomical yield and the resistance to disease and pest. The analyses of the data allowed the separation of the two basic species, *Saccharum spontaneum* and *Saccharum officinarum*, showed also two different genetic sources of *Saccharum spontaneum* clones. The use of the genetic similarity index in order to quantify polymorphism within and between the taxonomic units showed that the three basic *S. spontaneum*, *S. officinarum* and *S. robustum* were clearly distinct although the latter appear more closely related. The useful of the characterization of the genotypes by agronomic and sugar content traits and its genetic relationships by molecular markers (nuclear RFLP) was also shown.

The second work collection studied shown that *S. officinarum*, *S. spontaneum* and *Erianthus* spp. clones were distributed among three well differentiated groups in both nuclear and cytoplasmic levels. Within the *S. officinarum* group, clones were tightly clustered in nuclear and in cytoplasmic analysis in relation to other species. Three nuclear diversity subgroups can be considered among the clones studied. These clones were distributed by their cytoplasmic diversity in other three subgroups with a different clonal composition to the nuclear ones. The results obtained illustrated the low diversity among *S. officinarum* clones studied and the necessity to incorporate new materials, for instance, *Erianthus* spp. clones and wild clones (ECL) for improving cytoplasmic diversity. In the meanwhile, it can be taken a best profit of the genetic variation available, considering the diversity subgroups determined in this study when selecting *S. officinarum* as female parents by traditional methods.

The use RFLP and PCR amplifications by specific primers in the collection of wild forms of unknown origin studied, allow to determine the genetic relationship of seven ECL clones to *Erianthus* spp. and their higher similarity of the others ECL clones to *S. spontaneum* representatives. Considering the cytoplasmic diversity obtained in the Collection # 2, the occurrence of introgression and outcrossing in wild conditions among members of the *Saccharum* complex and the presence of highly favorable conditions to their diversification in the localities where they were collected was also evidenced.

The DNA polymorphism revealed in the core collection allowed the determination of groups based on their genetic diversity to assist their ulterior management for breeding purposes. In this sense, is very convenient take in account the cytoplasmic and nuclear (PCR amplification) pools detected in the ECL clones. An strategic option could be the use of the molecular characterization in the aim to recommend these clones for the nobilization program. In this sense, the results obtained suggest the exploitation of male parents in order to transfer useful agronomic characters such as profuse tillering, strong ratooning ability, wide adaptation and disease and pest resistance, presents in the wild species *S. spontaneum* and in *Erianthus* spp.. Similarly, in the case of female parents, the results obtained should allow breeders to select alternative cytoplasm by crossing with *S. spontaneum* and *Erianthus* spp. considering the agronomic performance of the clones which constitutes this collection.

The information obtained permits also the use of the RFLP profiles and the results of PCR test in the rapid identification of all the genotypes surveyed.

This Ph.D. Thesis is divided by five chapters related with Introduction, Bibliographic Analysis, Material and Methods, Results and Discussion, Conclusions and Recommendations. The document show also 21 tables, 10 figures, 251 references and 4 Anexos.

The scientific relevance of this thesis is based on its novel international information in relation with the nuclear and cytoplasmic diversity of the *Saccharum* complex and evidenced the occurrence of interspecific hybrids in wild conditions. A novel system to the identification of the sugarcane germplasm using the molecular markers reported here was designed. On the other hand, the collections studied, conserved in the germplasm bank of sugarcane, were characterized for the first time in Cuba by molecular markers and all the clones were taxonomically identified using the most powerful tools available.

The genetic pools determined in the collections permit also to assist the management of the clones for breeding purposes, and all the results obtained have been accepted and applied by the breeders of Sugarcane National Research Institute (INICA).