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Genetic diversity in sugarcane cultivars assessed by DNA markers and morphological traits

María F. Perera*, Marta E. Arias**, Diego D. Costilla***, Catalina Luque**, María B. García***, Josefina Racedo*, María I. Cuenya**, María P. Filippone* and Atilio P. Castagnaro*

ABSTRACT

Better knowledge of sugarcane genetic diversity will provide useful information concerning genotypic value for breeding programs and should help to improve the use and conservation of genetic resources and the protection of sugarcane varieties by intellectual property rights. Morphological descriptors are traditional tools to characterise varieties; however, they vary phenotypically because of environmental effects. Therefore, molecular markers have become increasingly important for identifying genotypes and estimating diversity, as they are accurate, readily available, and are not affected by the environment. The aim of this research was to evaluate genotypes used as parental materials in the Sugarcane Breeding Program of Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Argentina, by using molecular markers (AFLP and SSR) and morphological traits, and by comparing the data obtained with two statistical software programs (NTSys and InfoStat). All cultivars grouped in one main cluster of the dendrogram when using both programs and at least 150 data points. Local Argentine genotypes grouped together with US-varieties and no clear genetic differentiation was found, probably due to regular germplasm exchange. Although morphological traits reflected external resemblance only, the topology of the dendrogram was not modified when combining both molecular and morphological data. These results suggest that both characterisation methods should be used to estimate genetic diversity. Molecular markers should be included internationally for sugarcane variety protection.

Key words: breeding program, sugarcane germplasm bank, molecular markers, morphological descriptors.

RESUMEN

Diversidad genética de cultivares de caña de azúcar determinada por marcadores de ADN y caracteres morfológicos

Un mejor conocimiento de la diversidad genética de la caña de azúcar proveerá información útil sobre el valor de los genotipos para los programas de mejoramiento y contribuirá tanto a hacer un más eficiente uso y conservación de los recursos genéticos, como a asegurar los derechos de propiedad intelectual de los creadores de nuevas variedades. Si bien los descriptores morfológicos constituyen las herramientas más tradicionales para caracterizar a las variedades, pueden presentar variaciones fenotípicas causadas por factores ambientales. Por este motivo los marcadores moleculares son cada vez más importantes en la identificación de genotipos y la estimación de la diversidad, debido a su precisión, abundancia e independencia de factores ambientales. El objetivo de este trabajo fue evaluar genotipos empleados como padres en el Programa de Mejoramiento de la Caña de Azúcar de la Estación Experimental Agroindustrial Obispo Colombres (EEAOC), usando marcadores moleculares (AFLP y SSR) y caracteres morfológicos y comparando los datos obtenidos con dos programas informáticos estadísticos (NTSys e InfoStat). Todos los cultivares se agruparon en un mismo grupo con ambos programas, cuando se emplearon al menos 150 datos. Probablemente debido al intercambio regular de germoplasma, no se observó una clara diferenciación genética entre los genotipos locales y las variedades de los EE. UU., que se agruparon juntos. Aunque los caracteres morfológicos reflejan solamente la semejanza externa, la topología del dendrograma no se modificó cuando se combinaron datos moleculares y morfológicos. Estos resultados sugieren que ambos métodos de caracterización deberían ser utilizados para estimar la diversidad genética y que los marcadores moleculares deberían ser incluidos a un nivel internacional, para proteger las nuevas variedades de caña de azúcar.

Palabras clave: programa de mejoramiento, banco de germoplasma de caña de azúcar, marcadores moleculares, descriptores morfológicos.

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*Sección Biotecnología, EEAOC. franciscaperera@eeaoc.org.ar
**Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán.
***Sección Caña de Azúcar, EEAOC.

INTRODUCTION

In sugarcane breeding programs, parents showing high genetic diversity should be used in order to broaden the genetic base. In addition, exchange of genotypes occurs regularly among breeding programs in the world and, for these reasons, as well as for protecting new varieties, an accurate varietal identification is essential. Moreover, improved knowledge of sugarcane genetic diversity will contribute to a better use of genetic resources.

Molecular markers are powerful tools to estimate genetic diversity and to better understand the complexity of sugarcane genetics, as they are accurate, abundant and show no alterations caused by environmental factors. Morphological traits are useful for genetic evaluation, and the availability of a descriptor set, such as that proposed by the International Union for the Protection of New Varieties of Plants (UPOV), serves as an international standard for measuring genetic distances.

To date, and despite the importance of sugarcane production in Argentina, no major descriptive study has been carried out yet to identify genotypes and estimate their diversity. The aim of this study was to evaluate genotypes widely used as parents by the Breeding Program of Estación Experimental Agroindustrial Obispo Colombres (EEAOC), by employing different molecular techniques, studying morphological traits, and comparing two data analysis software packages for the correct interpretation of marker data.

MATERIALS AND METHODS

Sugarcane genotypes

Thirty-six genotypes, including 17 commercial cultivars from Argentina and the US as well as 19 varieties not yet commercially released, which are kept at the EEAOC and commonly used as parents in sexual crosses in the breeding program, were genotyped.

Molecular markers

DNA of each genotype was extracted from ground frozen leaves (Aljanabi *et al*., 1999). Fifteen SSR primers (Cordeiro *et al*., 2000; D´Hont, unpublished data), distributed in the sugarcane genome, and 16 AFLP primer combinations, selected based on the presence of scoreable and/or polymorphic bands (Vos *et al*., 1995), were used for genotype characterisation. Amplification products were separated in 6% polyacrylamide denatured gels by electrophoresis and bands were visualised using silver staining.

Morphological traits

In addition to molecular analysis, morphological characterisation was also performed by selecting eight genotypes, three of which (LCP 85-384, RA 87-3, and TUCCP 77-42) happen to be the most important commercial varieties in Tucumán. Another three (TUC 89-28, TUC 95-37 and TUC 97-8) were recently commercialised (2009), while the remaining two (TUC 97-7 and TUC 95-24) are clones at the final testing stage prior to a possible commercial release. All plant materials were characterised using 52 morphological characters proposed by UPOV (2005).

Data analysis

Each molecular and morphological allele was scored in a dominant manner and transformed into a 0 or 1 matrix. Genetic similarity was calculated by using the Jaccard coefficient. Cluster analyses were carried out by using two software packages: NTSys (Rohlf, 1993) and InfoStat (Di Rienzo *et al*., 2009).

RESULTS

AFLPs

The 16 primer combinations generated a total of 995 fragments, out of which 193 were polymorphic. Dendrograms obtained with the two software programs generated the same clusters. All cultivars grouped in one main cluster divided into at least seven subgroups, presenting a high similarity degree (0.94 to 0.99) (Figure 1). Dendrograms of the eight genotypes (selected for morphological analysis) were the same with both programs, and similarities ranged between 0.95 and 0.99.

SSRs

The 14 primer pairs produced 136 fragments, out of which 101 were polymorphic. Dendrograms showed the same clusters using both software packages. Related genotypes tended to group together, as well as those with the same origin. Genotype similarities ranged between 0.57 and 0.91. When the eight selected genotypes were grouped (0.57 to 0.82 similarity) and the two programs were compared, they did not show identical clusters. It was observed that when NTSys was used, cultivars from the same or related crosses tended to group together.

When both AFLP and SSR markers were used to generate dendrograms for the 36 genotypes and the eight selected genotypes, similarity ranged between 0.91 and 0.98, and 0.92 and 0.95, respectively, indicating very low genetic diversity.

Morphological traits

The eight genotypes varied greatly in several characters. However, they showed similarity in four characters (bud groove depth and length, by virtue of lacking a groove, pubescence on the bud, and size of underlapping auricle). Dendrograms obtained with both programs showed the same clusters (a 0.19 to 0.41 similarity).

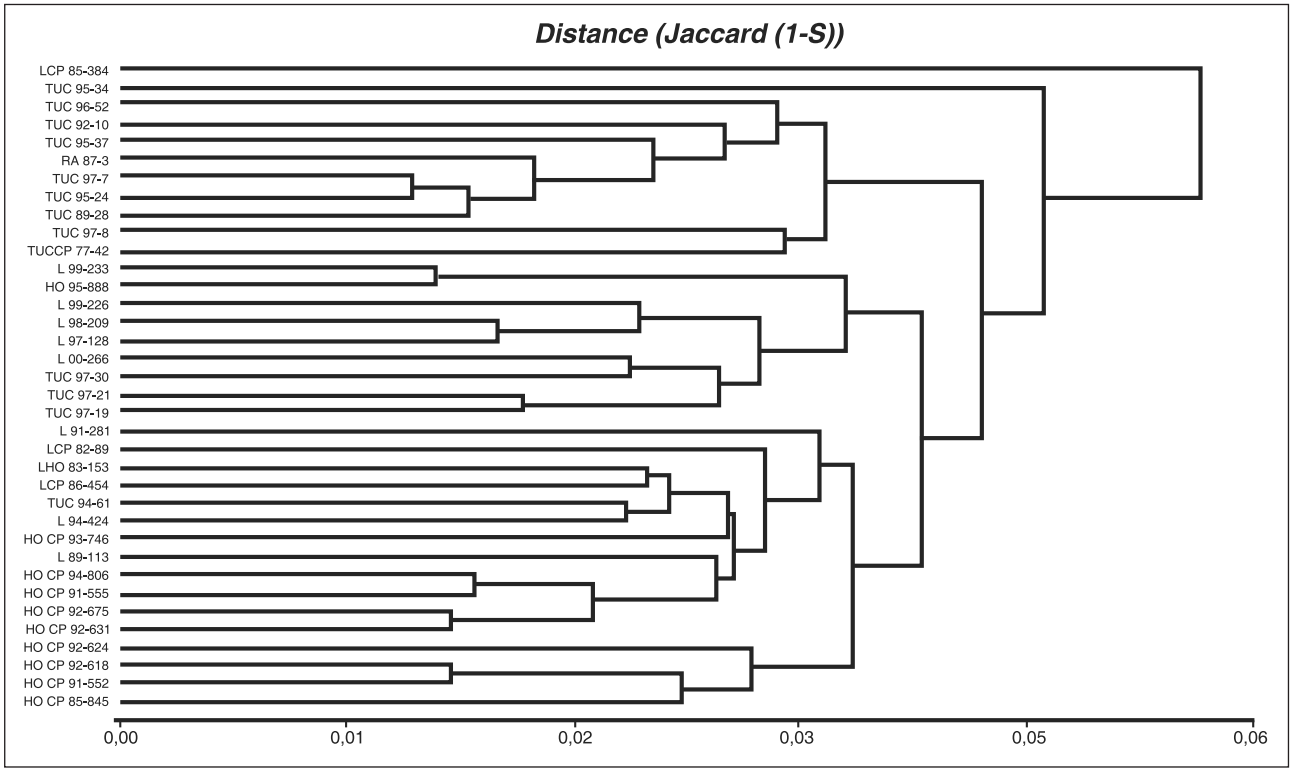

Figure 1. Dendrogram of 36 sugarcane genotypes based on the analysis of AFLP markers, by using InfoStat software.

Although morphological traits only revealed external genotype resemblance not directly associated with genetic relationships, when they were combined with molecular markers, the topology of the dendrogram obtained reflected genotype pedigree as when only molecular markers were used. In addition, genetic diversity could be estimated more accurately (0.86 to 0.90 similarity) (Figure 2).

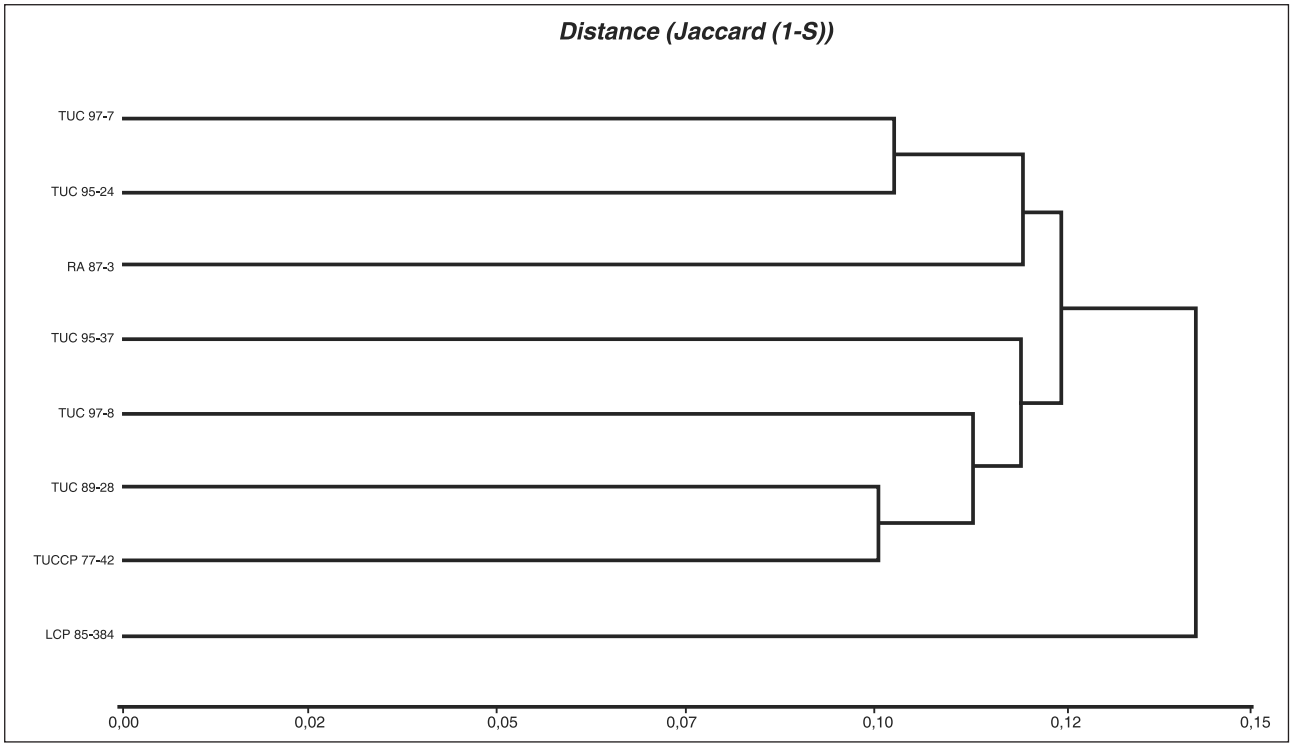

Figure 2. Dendrogram of eight selected sugarcane genotypes based on data from morphological traits, AFLP and SSR markers, by using InfoStat software.

InfoStat vs NTSys

Dendrograms obtained with both software programs did not always show the same clusters. Namely, when data number was high, the programs showed the same clusters, but when genotype or character numbers were reduced, dendrograms began to differ. Nevertheless, character numbers were more important than genotype numbers in determining the same clusters with both programs. Using AFLP data, the minimum character number needed to obtain the same cluster with both programs was 150 (Perera *et al*., 2012).

CONCLUSIONS

Both methods, morphological and molecular, should be used together to better estimate genetic diversity, and molecular traits should be included in the group of characters established and taken into account internationally for sugarcane variety protection and identification.

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